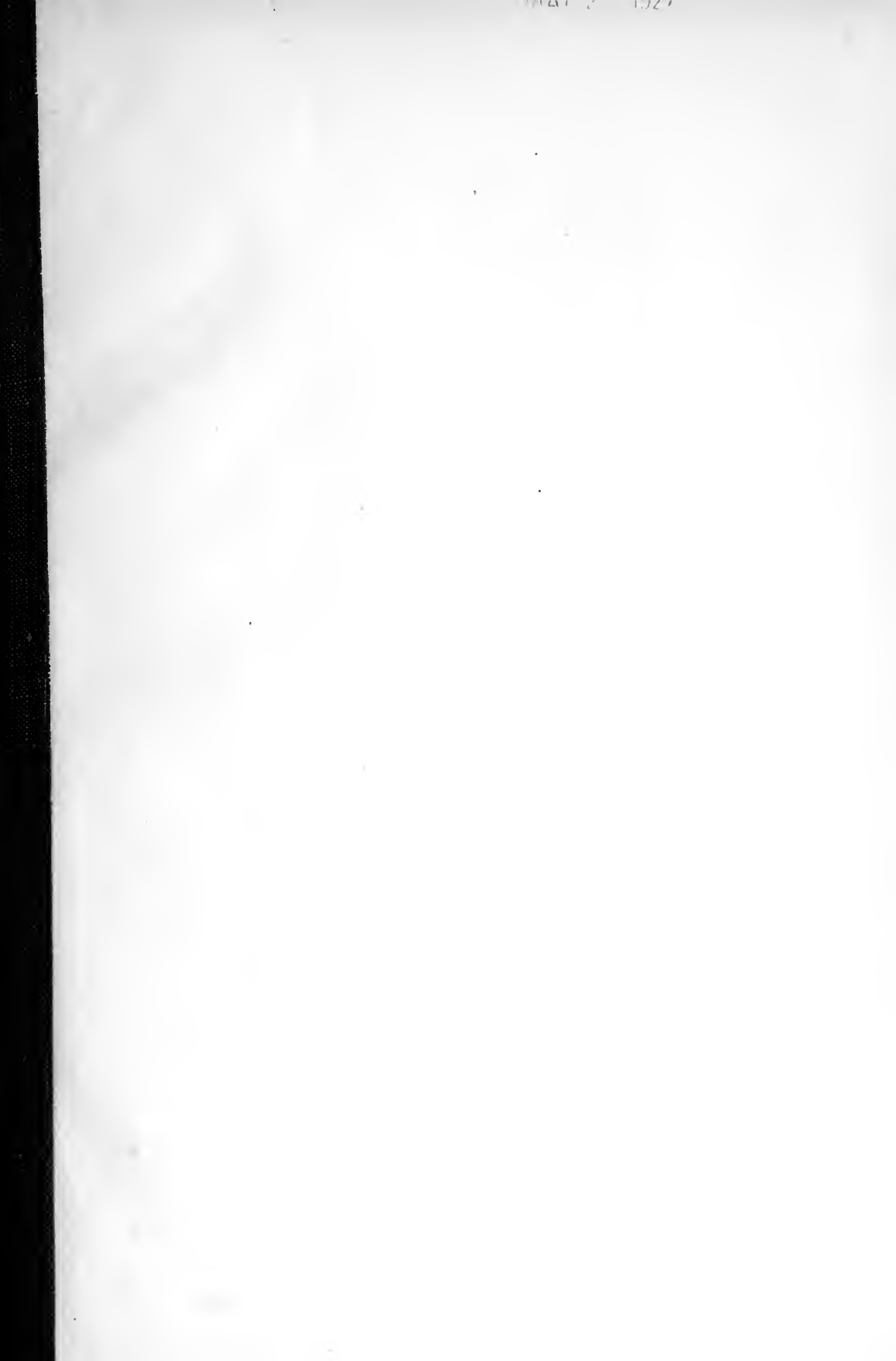


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THE JOURNAL OF HYGIENE

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ON THE CONSTRUCTION AND USE OF LIFE-TABLES FROM A PUBLIC HEALTH POINT OF VIEW.

By T. E. HAYWARD, M.B. (LOND.), F.R.C.S. ENG.

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THE time may be considered opportune for bringing forward this subject in that the near approach of the publication of the completely classified results of the census of 1901 will cause the attention of many Medical Officers of Health to be directed to the possibility and desirability of using the census data for working out Life-Tables for their respective districts, and doubtless the construction of many more local Life-Tables will be contemplated than was the case after the census of 1891.

Anything, therefore, which will tend to remove or diminish the initial difficulties associated with such an undertaking will probably be acceptable to those concerned.

It is desirable at the outset to clearly limit and define the scope of what it is proposed to attempt in this paper.

To enter into a full explanation of the mathematical theory upon which the construction of Life-Tables is based, or to give an account of all the possible different methods which may be employed, with a discussion of the reasons for adopting one or the other, would necessarily occupy more space, if not time, than that available.

It appears, therefore, to the writer that the most serviceable position which he can take up is that of one who having made a special study of this subject, and having as the result of much laborious experimental work arrived at certain definite conclusions as to what is the best and most accurate method to adopt, is desirous of giving to those who may be willing to accept it, guidance as to the process of constructing a Life-Table, in such a way that it may be followed without any more mathematical knowledge being presupposed than an acquaintance with the ordinary rules of Arithmetic and with the use of common logarithms. With these considerations in view it is proposed

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(1) To describe the construction of a complete or "extended" Life-Table (*i.e.* one for every separate year of life) by means of an "analytical" method.

(2) To give a short account of the practical uses to which such a Life-Table may be put when constructed.

(3) To show how by certain simple modifications of the "short" method first devised by the late Dr Wm. Farr, results can be arrived at which are practically identical with those worked out by the much more laborious extended method.

I. CONSTRUCTION OF AN EXTENDED LIFE-TABLE.

Data required.

The first and by no means the least important part of Life-Table construction is the compilation of the necessary foundations of numerical facts. These are the following:

(1) The total numbers of population as enumerated at two successive censuses, say 1891 and 1901.

These are required to be for each sex classified into the following age-groups:

0—5	25—35	65—75
5—10	35—45	75—85
10—15	45—55	85 and upwards
15—25	55—65	

It is better to take the age-group 15—25 together rather than in two groups as 15—20 and 20—25.

As the data after age 85 are unreliable it is best *not* to make a separate age-group of 85—95.

It may also be requisite in those districts within which are situated large public institutions, such as Hospitals or Lunatic Asylums, to correct the census numbers.

(2) The numbers of deaths registered in the district during the ten calendar years most nearly corresponding to the interval between the two censuses, say 1891—1900, classified into age-groups for each sex corresponding to those above given for the population-numbers.

The deaths of the age-group 0—5, however, require to be sub-classified as follows:

0—6 months	1—2 years
6—12 months	2—3 "
Total under 1 year	3—4 "
	4—5 "

It is necessary to take all possible care in getting the accurate numbers of deaths, by *excluding* all deaths of persons not properly belonging to the district, and also *including*, so far as can be ascertained, all deaths of persons properly belonging to the district which have occurred outside it, in Workhouses, Hospitals, &c. It may be noted in passing that it is greatly to be desired that the Registration arrangements in this country should be so amended as to facilitate these corrections.

It must be realized that an error of *one* in the death-number will have a very much greater effect in producing incorrect results than an error of *ten* in the population-number.

Thus, from the formulæ to be shortly given it may be easily shown that with a population-number of 1000, and a death-number of 10, increasing the latter by one has the same effect as decreasing the former by 91, and decreasing the death-number by one is equivalent to *increasing* the population-number by 111.

(3) It is also requisite to have the following returns of deaths for some years preceding the decennial period.

Deaths at age 0—1 for each of the years 1887—90 inclusive				
"	"	1—2	"	"
"	"	2—3	"	"
"	"	3—4	for the year	1890

(4) The numbers of male and female births in each of the years 1886—1900 inclusive are also required.

How to calculate mean population-numbers from the census data.

After compiling and tabulating the data the next thing required is to deduce from the numbers enumerated at the two successive censuses such numbers as shall truly express the mean numbers living during the ten calendar years. In other words we have to calculate the "years of life" or the "lives at risk" during the decennium. This necessity has to be considered as applying (1) to the total population-numbers, and (2) to the numbers of the separate age and sex groups.

(a) The most simple and obvious method would be to take the arithmetical means of the two successive census enumerations. If this were done the sums of the parts, *i.e.* the separate age-groups, would necessarily equal the whole, *i.e.* the total population-number.

Although this method was used by the late Dr Wm. Farr, for the calculation of decennial death-rates, it cannot be considered accurate enough for Life-Table purposes for two reasons:

(1) On the assumption that population varies in the direction

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of increase or decrease at a constant "rate," that is in Geometrical Progression, the true mean must necessarily be less than the arithmetical mean.

(2) Since the interval between two censuses does not exactly correspond with ten calendar years, but begins and ends a fourth part of a year *later*, the arithmetical mean will give a result *too great* in an *increasing* population and *too little* in a *decreasing* population. (See *Supplement to Fifty-fifth Annual Report of Registrar-General*, pp. xlii and xliii.)

(b) Admitting then the principle of Geometrical Progression, there are certain difficulties to be met with in its application. The chief difficulty is that the *sum of the parts cannot be made to coincide with the whole*, that is to say, the number arrived at by applying a process of calculation to the whole population-numbers differs from the sum of the numbers obtained by applying the same process to the numbers of the separate age and sex groups.

This difficulty has usually been overcome by making the *sum of the parts to be the true whole*.

Thus in working out the Brighton Life-Table (see Dr Newsholme's *Vital Statistics*, Third Edition, p. 263) each age-group was dealt with separately.

P_1 being the number in an age-group enumerated at the census of 1881, and P_2 being the number for the same age-group enumerated at the census of 1891, then $\frac{P_2}{P_1} = r$, and the years of life for that age-group are taken as the sum of the ten mid-year populations worked out by summing the series

$$P_1 \cdot r^{\frac{1}{10}} + P_1 \cdot r^{\frac{5}{10}} + \dots + P_1 \cdot r^{\frac{37}{10}}$$

the sum of the series being $P_1 \cdot \frac{r^{\frac{1}{10}} (r - 1)}{r^{\frac{1}{10}} - 1}$.

The total years of life were taken as the sum of the results of applying this method to each separate age-group. (This is not precisely the method given by Dr Newsholme, as he has given the calculation in two stages.) However, the sum of the ten mid-year populations is only an approximation to the true years of life which are to be obtained by the formula $\left(P_1 \cdot \frac{r - 1}{r^{\frac{1}{10}} \cdot \log_e r} \right) \times 10$.

This practically was the method used by Dr Farr in his *English Life-Table*, p. xviii., applied to each age-group and the sum of the parts was taken as the true total.

Now, if there be any truth in the principle of geometrical progression at a constant rate applied to population-numbers, it would seem more rational to apply the principle to the total population-numbers and to devise some way of making the sum of the parts correspond to the whole.

The method which has been described by the writer in the *Journal of the Royal Statistical Society*, vol. LXII., Part iii., pp. 449—451, and also in Dr Newsholme's *Vital Statistics*, Third Edition, pp. 280—281, is based on the principle of first working out the true mean total population-number and then dividing this up by the method of mean proportions, which assumes that from one census to the next the proportion of the separate parts to the whole is changing uniformly in arithmetical progression, and in which the true mean total is finally divided up according to the proportions existing on the above assumption at $4\frac{3}{4}$ years after the earlier census, *i.e.* at the exact middle of the ten calendar years.

This is what until recently has appeared to the writer to be the best method, but it is defective in that it assumes that the proportion of the part to the whole taken at *one* point, is the true mean proportion for the *whole* period, and it gives identical results on reversing the proportions of the part to the whole at the two censuses.

The writer has been recently indebted, however, to Mr A. C. Waters for the knowledge of a more perfect method which he has worked out by an application of the Integral Calculus, and by which the true mean is arrived at by means of a full mathematical expression of the two assumptions, (1) that the *whole* population-number is changing in geometrical progression at a constant "rate," and (2) that the proportion of any selected part to the whole is uniformly changing in arithmetical progression.

As Mr Waters has since published a paper on his method in the *Journal of the Royal Statistical Society*, vol. LXIV., Part ii., June 1901,

of any one district, but it is intended to be used on the assumption that the *whole* which is changing in Geometrical Progression is the population of the *entire country*, and that the *parts*, the proportions of which to the whole are changing in Arithmetical Progression, are the total populations of separate districts, as well as the sub-groups into which these latter are divided.

If Q_1 be the total population of the whole country (England and Wales) at the earlier census, and Q_2 the total population at the later census, then $\frac{Q_2}{Q_1} = r$, and the true mean total for the ten calendar years will be $Q_1 \cdot \frac{r-1}{\frac{1}{r^{10}} \cdot \log_e r}$.

(The "years of life" will be obtained by multiplying the last expression by 10 as the unit of calculation is a decennium.)

Now it can be shown that, on the two assumptions indicated above, two constant multiplying factors, m and n , can be worked out so that if P_1 be some selected part of Q_1 , whether the total population of a district or some subdivision or age-group belonging to it, and if P_2 be the corresponding part of Q_2 , then the true "years of life" for that part will be $(m \cdot P_1 + n \cdot P_2) \times 10$.

It is obvious that the *sum of all the separate parts* treated in this way must be equal to $(m \cdot Q_1 + n \cdot Q_2) \times 10$.

The only data needed for working out m and n are the total population-numbers for the whole country at two successive censuses; these being Q_1 and Q_2 respectively and r being $\frac{Q_2}{Q_1}$,

$$\text{then} \quad m = \frac{(r-1) \left(\frac{41}{40} + \frac{1}{\log_e r} \right) - r}{\frac{1}{r^{10}} \cdot \log_e r},$$

$$\text{and} \quad n = \frac{r - (r-1) \left(\frac{1}{40} + \frac{1}{\log_e r} \right)}{r \cdot \frac{1}{r^{10}} \cdot \log_e r}.$$

If the above values of m and n be substituted in the expression $m \cdot Q_1 + n \cdot Q_2$ (i.e., $m \cdot Q_1 + n \cdot rQ_1$), and then simplified, the result will be found to work out to $Q_1 \cdot \frac{r-1}{\frac{1}{r^{10}} \cdot \log_e r}$.

(In order to work out these constants with the greatest possible degree of accuracy the values of r and of the hyperbolic logarithm of r have to be obtained to a large number of decimal places.)

When the final results of the census of 1901 are made known it will be possible for Medical Officers of Health to obtain the values of m and n without the trouble of working them out for themselves, as they will doubtless be published¹, and then the calculation of true mean numbers or years of life will be reduced to a very simple and easy matter.

To illustrate this the process may be shown as applied to an instance taken from the census enumerations of 1881 and 1891.

If reference be made to Dr Newsholme's *Vital Statistics* at page 262 the census data will be found on which his Life-Table for Brighton was based.

We find there,

Total population for males and females at census of 1881	= 128,350
" " " " " "	1891 = 141,970

These two numbers have to be considered as forming parts of the entire census populations of the whole of England and Wales. Now the factors m and n for England and Wales, censuses 1881 and 1891 (the responsibility for their accuracy being the writer's) are

$\log m = \bar{1} \cdot 7354639$	$m = \cdot 54383$
$\log n = \bar{1} \cdot 6600871$	$n = \cdot 45718$

Therefore the years of life for Brighton during the ten years 1881—90

$$= \{(\cdot 54383 \times 128,350) + (\cdot 45718 \times 141,970)\} \times 10.$$

Since	$\log m + \log 128,350 = \log (m \times 128,350)$
i.e.	$\bar{1} \cdot 7354639 + 5 \cdot 1083959 = 4 \cdot 8438598$
\therefore	$m \times 128,350 = 69,800 \cdot 7,$
and since	$\log n + \log 141,970 = \log (n \times 141,970)$
i.e.	$\bar{1} \cdot 6600871 + 5 \cdot 1521966 = 4 \cdot 8122837$
\therefore	$n \times 141,970 = 64,905 \cdot 8$

and the total years of life = $(69,800 \cdot 7 + 64,905 \cdot 8) \times 10 = 1,347,065.$

¹ Based on the total population for England and Wales given in the Preliminary Report of the recent census, the factors " m " and " n " for the censuses of 1891 and 1901 have been worked out as follows :

$$m = \cdot 5445944 \quad \log m = \bar{1} \cdot 7360732$$

$$n = \cdot 4564973 \quad \log n = \bar{1} \cdot 6594383$$

(see Mr Waters' paper in the *Journal of the Royal Statistical Society* already referred to).

It is not likely that the finally corrected census number for 1901 will vary sufficiently from the number as yet given, to cause any material error through using the above values of m and n .

This number exceeds by 531 the number as worked out by Dr Newsholme.

By dealing similarly with the two census numbers for each of the age-groups the years of life belonging to each will be obtained, and the results will be checked by finding that the sum of the parts exactly corresponds with the total as already found.

It will now be evident that when once the factors m and n are obtained this method is to be preferred not only for its mathematical accuracy but for its simplicity and ease in application.

*On the calculation of p_x values and on the relation
between p_x and m_x .*

Having now obtained and set down in tabular form the years of life and the deaths for each of the age and sex groups, the use which has to be made of these numbers is to calculate by their means the series of fractions which are set down in a Life-Table under the heading p_x , and which may be regarded as the most essential part of it. To work out these fractions for every single year of life constitutes by far the most laborious and difficult part of the task of constructing an extended Life-Table.

In Life-Table notation p_x simply means the chance (or probability) of surviving from the exact age x to the exact age $x + 1$.

Thus if we have any number of persons l_x , of exact age x living at the beginning of a calendar year, and if a certain number d_x die during the year, then the chance of any individual of the l_x persons surviving to the end of the year is expressed thus

$$p_x = \frac{l_x - d_x}{l_x} = \frac{\text{survivors at end of year}}{\text{no. living at beginning of year}}.$$

We shall not be able, however, to deduce from our data the required p_x values in quite so simple a way, seeing that the population-numbers enumerated at the census and the deaths returned in the death-registers do not give us the numbers at exact age x , but the numbers at all ages between the fixed points x and $x + n$.

If then we have any number of persons P_x enumerated or estimated as living at the middle of a calendar year, this means that they may be of any age from x to $x + 1$, and if a certain number d_x are returned as dying during the year who also may be of any age from x to $x + 1$, the problem of calculating p_x is more complicated than the simple case previously considered.

In order to solve it we may assume two things—

(1) that at the middle of the calendar year the average age of the P_x persons was $x + \frac{1}{2}$, and

(2) that the number of deaths has been evenly distributed during the year, in other words that they have happened at equal intervals, half occurring in the first half of the year and half in the latter half of the year.

Therefore on these two assumptions, which when large numbers are being dealt with may be considered to be approximately true for every year of life *except the first*, the number living at the *beginning* of the year must be $P_x + \frac{1}{2}d_x$, and the number surviving at the *end* of the year must be $P_x - \frac{1}{2}d_x$ and therefore

$$p_x = \frac{P_x - \frac{1}{2}d_x}{P_x + \frac{1}{2}d_x} = \frac{2P_x - d_x}{2P_x + d_x}.$$

In actual practice the population and death-numbers are given in age-groups x to $x+n$, and if we are dealing with these groups as a whole it is assumed that the average age at the middle of the calendar year is $x + \frac{1}{2}n$. However, by certain processes of calculation the groups may be so divided up into numbers corresponding to each separate year of life that the problem is reduced to what has been just given.

It has been usual in Life-Table calculations to obtain the p_x values not *directly* from the population and death-numbers, but *indirectly* by first calculating m_x values. Now m_x is the "rate of mortality" per unit of the number living during the age x to $x+1$, and it is expressed by the fraction $\frac{d_x}{P_x}$. It is called in actuarial terminology the "central death-rate," meaning the rate at which people are dying at the central point of the age x to $x+1$.

We have already found that $p_x = \frac{P_x - \frac{1}{2}d_x}{P_x + \frac{1}{2}d_x}$, and dividing the numerator and denominator of this fraction by P_x , we have

$$p_x = \frac{\frac{P_x - \frac{1}{2}d_x}{P_x}}{\frac{P_x + \frac{1}{2}d_x}{P_x}} = \frac{1 - \frac{1}{2}\frac{d_x}{P_x}}{1 + \frac{1}{2}\frac{d_x}{P_x}} = \frac{1 - \frac{1}{2}m_x}{1 + \frac{1}{2}m_x} = \frac{2 - m_x}{2 + m_x}, \text{ since, as we have seen, } m_x = \frac{d_x}{P_x}.$$

$$\text{Conversely, } m_x = \frac{2(1 - p_x)}{1 + p_x}.$$

If we are making calculations to deduce the "Law of mortality" from the observed facts with relation to the living and the dying at certain ages, the m_x values are necessary, but for the ordinary work of

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Life-Table construction, such as is now being considered, and which is based on a *particular series of observed facts* without any necessary regard to other similar series of facts, there is no need to work out these values.

Calculation of the p_x values for the first five years of life.

Although at each census there are enumerated the numbers of those living at each of the first five years of life, *i.e.* at ages 0—1, 1—2, 2—3, 3—4, and 4—5, the results have hitherto been found, by reason of obvious misstatement of age, to be altogether impossible and unreliable, and they have to be discarded in Life-Table construction.

The only use which can be made of the census data for these five years is to give us the total number for each sex in the age-group 0—5, and from this, by means of the process of calculation already described, the mean number living, or the “years of life” can be deduced for this age-period.

Under these circumstances an approximate calculation has to be made based on the recorded numbers of births and deaths.

The principle of this will be more readily comprehended if the process be first of all considered with relation to one single calendar year.

The deaths under one year of age (at age 0—1) which were registered in the year 1891 occurred partly out of those born in 1890 and partly out of those born in 1891. It may therefore be a fair assumption to consider that these deaths occurred out of (1) either the births registered during the last half of 1890 *plus* those registered in the first half of 1891, or (2) more conveniently out of a number represented by the arithmetical mean of the births registered in the two years 1890—91.

For the purposes of Life-Table construction we have to consider that on 1st January, 1891, there existed *at birth* a number corresponding to half the sum of the births in 1890 and 1891, and that the deaths at age 0—1 which were registered during the year 1891 occurred out of these.

In order to get the mean number living at age 0—1 for the year 1891, or in other words the number living at the middle of the year 1891, we shall have to deduct from the number at birth on 1st January the deaths under 6 months of age which were registered in that year.

If we call this mean number living at age 0—1 P_0 , then the chance of surviving from age 0 to age 1 will be represented by

$$\frac{P_0 - \text{deaths at 6 to 12 months}}{P_0 + \text{deaths at 0 to 6 months}} = \frac{\text{survivors at end of year}}{\text{number at birth}}.$$

It would of course come to the same thing if the p_0 fraction were represented thus

$$\frac{\text{number at birth} - \text{deaths at age 0 to 1}}{\text{number at birth}}.$$

Similarly half the sum of the births in 1889 and 1890 represents the number *at birth* on 1st January, 1890, and after deducting the deaths at age 0—1 during 1890 we shall have the number *at age 1* surviving on January 1st, 1891 out of whom the deaths at age 1—2 will occur during 1891. The mean number living at age 1—2 at the middle of 1891 will be found by deducting half the deaths at age 1—2 occurring during 1891, and calling the resulting number P_1 then the chance of surviving from age 1 to age 2, *i.e.* p_1 , will be represented by

$$\frac{P_1 - \frac{1}{2} \text{ deaths at age 1 to 2}}{P_1 + \frac{1}{2} \text{ deaths at age 1 to 2}} = \frac{\text{survivors at end of year}}{\text{survivors at beginning of year}}.$$

It would again come to the same thing to represent p_1 as being equal to

$$\frac{\text{number at age 1} - \text{deaths at age 1 to 2}}{\text{number at age 1}}.$$

Still calculating on the same principle, half sum of births in 1888 and 1889, less deaths at age 0—1 in 1889, and less deaths at age 1—2 in 1890, will give the number at age 2 living on 1st January, 1891, out of whom the deaths at age 2—3 will occur during 1891.

Commencing with $\frac{1}{2}$ sum of births in 1887 and 1888 by a similar method we arrive at the number of survivors at age 3 on 1st January, 1891, out of whom the deaths at age 3—4 will occur during 1891.

Finally by commencing with $\frac{1}{2}$ sum of births in 1886 and 1887 and deducting in succession deaths at age 0—1 during 1887, deaths at age 1—2 during 1888, deaths at age 2—3 during 1889, and deaths at age 3—4 during 1890, we obtain the number *at age 4* surviving on 1st January 1891 out of whom the deaths at age 4—5 will occur during 1891.

The mean numbers living P_2 , P_3 , and P_4 and the expressions representing p_2 , p_3 , and p_4 , are arrived at in the same way as P_1 and p_1

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given above, the assumption being that for every year of life *except the first*, the deaths occur at equal intervals during the calendar year.

If the year 1892 be similarly dealt with and then every year in succession until and including 1900, and if finally the results are summed, the following scheme will be arrived at for the decennium 1891—1900.

- (a) For the number *at Birth*
 $\frac{1}{2}$ births in 1890 + all births in 1891 to 99 + $\frac{1}{2}$ births in 1900.
- (b) For the number *at 1 year of age*
 $\frac{1}{2}$ births in 1889 + all births in 1890 to 98 + $\frac{1}{2}$ births in 1899
less deaths at age 0—1 in the ten years 1890—99.
- (c) For the number *at 2 years of age*
 $\frac{1}{2}$ births in 1888 + all births in 1889 to 97 + $\frac{1}{2}$ births in 1898
less { deaths at age 0—1 in the ten years 1889—98
 " " 1—2 " " 1890—99.
- (d) For the number *at 3 years of age*
 $\frac{1}{2}$ births in 1887 + all births in 1888 to 96 + $\frac{1}{2}$ births in 1897
less { deaths at age 0—1 in the ten years 1888—97
 " " 1—2 " " 1889—98
 " " 2—3 " " 1890—99.
- (e) For the number *at 4 years of age*
 $\frac{1}{2}$ births in 1886 + all births in 1887 to 95 + $\frac{1}{2}$ births in 1896
less { deaths at age 0—1 in the ten years 1887—96
 " " 1—2 " " 1888—97
 " " 2—3 " " 1889—98
 " " 3—4 " " 1890—99

Let the five numbers deduced by the above scheme be denoted respectively as *a*, *b*, *c*, *d*, and *e*.

In order to obtain from them the sum of the ten mid-year or mean population-numbers,

from <i>a</i> subtract	deaths	at age	0—6 months	during	1891—1900		
" <i>b</i>	"	$\frac{1}{2}$	"	"	1—2 years	"	"
" <i>c</i>	"	$\frac{1}{2}$	"	"	2—3 " "	"	"
" <i>d</i>	"	$\frac{1}{2}$	"	"	3—4 " "	"	"
" <i>e</i>	"	$\frac{1}{2}$	"	"	4—5 " "	"	"

Let the five altered numbers thus obtained be denoted respectively by n_0 , n_1 , n_2 , n_3 , and n_4 , and let their sum = N .

Now N represents the sum of the ten mid-year population-numbers of those at all ages from birth to age 5.

We have previously obtained from the census data a mean population-number of those at all ages from birth to age 5, and the

multiplication of this by 10 gave us the "years of life" or the total number of those living at ages 0—5 during the decennium. Let this latter number be denoted by C .

Were it not for disturbing influences, N and C ought to at least very nearly correspond. However as a matter of fact N is usually found to be the greater¹. The principal causes of this difference are (1) excess of emigration over immigration and (2) over-statement of age in the death-registers.

However, we must take the total C as a fixed quantity, and divide it up in the proportions which n_0, n_1, n_2, n_3 and n_4 respectively bear to N , the resulting numbers being P_0, P_1, P_2, P_3 and P_4 .

Thus	$n_0 : N :: P_0 : C,$
or	$\log P_0 = \log n_0 + (\log C - \log N),$
similarly	$\log P_1 = \log n_1 + (\log C - \log N),$
	&c. &c.

Then	$P_0 = \frac{P_0 - \text{deaths at 6 to 12 months during 1891 to 1900}}{P_0 + \text{deaths at 0 to 6 months during 1891 to 1900}},$
	$P_1 = \frac{P_1 - \frac{1}{2} \text{ deaths at age 1 to 2 during 1891 to 1900}}{P_1 + \frac{1}{2} \text{ deaths at age 1 to 2 during 1891 to 1900}},$
	&c. &c.

The distribution of the difference between N and C in proportion to n_0, n_1, n_2, n_3 and n_4 , is open to the objection that migration or other disturbing influences may have not existed actually in quite the same proportions. However, after a good deal of labour expended in trying to find a better way the writer is of opinion that the uncertainty of the various disturbing factors is too great to justify any departure from the method which has usually been adopted.

In case any reader should note a difference between the above description and what has previously been given by the writer (see *Journal of the Royal Statistical Society*, September, 1899, and Dr Newsholme's *Vital Statistics*, Third Edition, pp. 271—273), it simply amounts to this—formerly N and C were made *comparable* by *adding* deaths to C —now they have been made comparable by *subtracting* deaths from N .

¹ The data for England and Wales for 1841—50 and 1851—60 produce a value of N less than C , the difference being most marked in 1841—50. This is probably explained by the births having not all been registered. For each succeeding decennium up to 1881—90 there has been a progressively increasing excess of N over C .

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For the sake of future convenience in calculating, the p_0 value may be taken in two stages, thus:

$$(1) \quad p_{0 \text{ to } \frac{1}{2}} = \frac{P_0}{P_0 + \text{deaths at 0 to 6 months}},$$

$$(2) \quad p_{\frac{1}{2} \text{ to } 1} = \frac{P_0 - \text{deaths at 6 to 12 months}}{P_0}.$$

How to calculate the p_x values from p_5 onwards.

Up to the point which has now been reached the work is the same for a short or extended Life-Table, or whether the "graphic" or an "analytical" method is to be afterwards followed.

(For a description of the "graphic" method reference may be made to Dr Newsholme's *Vital Statistics*, as the present writer has only undertaken to try to explain an analytical method.)

The data available in either case are the "years of life," *i.e.* ten times the mean annual population-number, and the deaths for the ten calendar years, as classified in the given age-groups.

It is obvious that a mean value of $p_{x \text{ to } x+n}$ might be easily calculated for each age-period by the formula

$$\frac{2P_{x \text{ to } x+n} - d_{x \text{ to } x+n}}{2P_{x \text{ to } x+n} + d_{x \text{ to } x+n}}.$$

As will be shown afterwards this is the plan upon which the short method of Life-Table construction is based, but for an extended Life-Table it is necessary to adopt some process of calculation so that the p_x values when plotted out to scale shall show not a series of step-like abrupt ascents or descents, as would be the case by the simple method just referred to, but an even curve without any sudden transitions or breaks in its symmetry.

Such a curve when obtained may not be exactly such as the true facts for each separate year of life would show (if we could get them), for some age-periods are more "critical" to life than others. However, it is the nearest approach which we can make to the hypothetical true curve and probably does not diverge very greatly from it.

The "analytical" method consists in deducing from the given numerical facts the required p_x values by means of some adaptation of the mathematical process known as "Interpolation" in a series of quantities by the method of "Finite Differences."

On "Finite Differences" and Interpolation.

As it would scarcely be possible for anyone to calculate by this process without having at least some general idea, clear and precise in so far as it goes, of its essential principles, some preliminary attempt must be made to explain these, in spite of the limitations laid down at the commencement of this paper.

Suppose that we take a series of numerical quantities, for example's sake the fourth powers of the numbers 3, 4, 5, 6, and 7, and set them down in inverse order, as in the left-hand column below, marking them in succession by the symbols u_0 , u_1 , u_2 , u_3 , and u_4 .

		Δ	Δ^2	Δ^3	Δ^4
u_0	2401	-1105	+434	-132	+24
u_1	1296	-671	+302	-108	
u_2	625	-369	+194		
u_3	256	-175			
u_4	81				

Now let this column of figures be "differenced," that is, change the sign of the upper quantity, and take the algebraical sum of it and the one below, that is their *sum if the signs are like*, and their *difference if the signs are unlike*. Let this be done in succession all down the column. In the next column to the right we have now the series of "first differences," marked by the symbol Δ . The first difference opposite u_0 is called Δu_0 , and the first difference opposite u_1 is called Δu_1 , etc., etc. Let a similar operation be carried out with the column of first differences, then we get the "second differences," marked as Δ^2 , and the one in a line with u_0 is called $\Delta^2 u_0$. Let the process be repeated until we come to the last difference Δ^4 , beyond which the process cannot be carried.

We have thus a series of five quantities giving a "constant fourth difference," or "with four orders of differences," and generally for n orders of differences we must have $n+1$ terms.

Now several considerations will at once be obvious.

(1) Having had given the five quantities u_0 , u_1 , u_2 , u_3 , and u_4 we have obtained by successive differencing the values of Δu_0 , $\Delta^2 u_0$, $\Delta^3 u_0$, and $\Delta^4 u_0$, but if we had had given u_0 and the line of differences *opposite to* u_0 we could just as easily have carried these differences down so as to have obtained the values of u_1 , u_2 , u_3 , and u_4 .

Thus adding Δu_0 , *i.e.* -1105, to u_0 , *i.e.* 2401, we obtain u_1 . *i.e.* 1296.

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Again, adding $\Delta^2 u_0$, *i.e.* + 434, to Δu_0 , *i.e.* - 1105, we obtain Δu_1 , *i.e.* - 671, and adding this to u_1 we obtain u_2 , etc., etc.

The most convenient way to proceed, having given $u_0 = 2401$, $\Delta u_0 = -1105$, $\Delta^2 u_0 = +434$, $\Delta^3 u_0 = -132$, and $\Delta^4 u_0 = +24$, is shown as follows, since it is easier to work from left to right.

			+ 2401 = u_0
			- 1105
		- 1105	+ 1296 = u_1
		+ 434	- 671
	+ 434	- 671	+ 625 = u_2
	- 132	+ 302	- 369
	+ 302	- 369	+ 256 = u_3
- 132	- 108	+ 194	- 175
+ 24	+ 194	- 175	+ 81 = u_4
- 108			

(2) By carrying down the differences in the above table one stage further, *i.e.* by continuous *addition* of the successive differences, the value of another term, u_5 , could be obtained, which will be found to be 16, the fourth power of the number 2, and so on for as many terms as we please, all the terms of the series having the constant fourth difference + 24.

On the other hand by successive *subtraction* of the differences, as set down in the table first given opposite u_0 , *i.e.* by changing their signs and adding, we could carry the series one stage *upwards* and obtain the term u_{-1} , which will be found to be 4096, *i.e.* the fourth power of the number 8, and so on ad infinitum.

The general equation which expresses the relation of the terms of a series of quantities differing from each other by a constant n th difference is the following, which follows the law of the "Binomial Theorem."

$$u_x = u_0 + x \Delta u_0 + \frac{x(x-1)}{2} \Delta^2 u_0 + \frac{x(x-1)(x-2)}{2 \cdot 3} \Delta^3 u_0 + \frac{x(x-1)(x-2)(x-3)}{2 \cdot 3 \cdot 4} \Delta^4 u_0 + \dots$$

By means of this equation any term u_x of the series can be calculated without the trouble of working up or down to it by the method above indicated. This may be verified by being applied to the numerical instances already used.

If working upwards, *i.e.* if the value of x is negative, care is needed with regard to the signs.

Again, if the above equation be expanded and reduced to its simplest

form, it will be found that each power of x has a *constant coefficient*—that is the equation can be expressed in the following form

$$u_x = A + Bx + Cx^2 + Dx^3 + Ex^4 + \dots$$

Thus B , or the coefficient of $x = \Delta u_0 - \frac{1}{2} \Delta^2 u_0 + \frac{1}{3} \Delta^3 u_0 - \frac{1}{4} \Delta^4 u_0 + \dots$

Certain formulae will afterwards be given the use of which is to obtain the line of differences opposite to u_0 . If the object of the above brief remarks had been to explain *how* these formulae had been *worked out* they would have had to be expanded much beyond the limits laid down. It may be possible, however, to *work from* them with such explanation as has been given.

The process of interpolation may be applied to the population and death-numbers in such a way as to obtain a value of $2P - d$ and $2P + d$ for each year of age and therefore the value of p_x by the fraction

$$\frac{2P - d}{2P + d}$$

The chief objection to this method is that it entails the labour of a *double* series of interpolations all throughout.

Another method is to first work out p_x values at certain fixed ages, $p_5, p_{10}, p_{15}, p_{25}$, etc. and then by applying the formulae of interpolation to these to obtain the required complete series of p_x values.

Such a method is the one proposed to be now explained. It is based on an application of the Differential Calculus devised originally by Mr A. C. Waters. A full description of this, with some proposed slight modifications, is given in the *Journal of the Royal Statistical Society*, for December 1900, to which any reader is referred who may be desirous of going into the mathematical theory.

In this communication the terminology of the Differential Calculus will be eliminated and only such explanations will be attempted as may suffice for the arithmetical work required.

In order to demonstrate the method of procedure it will be necessary to take some assumed set of foundation figures.

In the two left-hand columns given below we are supposed to have the "years of life" and the deaths in ten calendar years at the age-periods indicated for males.

From these are constructed the two right-hand columns representing twice population (*i.e.* years of life) minus deaths, and twice population plus deaths, *at age x and upwards*.

The reason for constructing the two right-hand columns which represent population and deaths at age x and upwards will be com-

At age	Years of life	Deaths	At age and upwards	$2P-d$	$2P+d$
4—5	330,090	5,645	4	23,608,335	24,015,655
5—10	1,571,715	11,980	5	22,953,805	23,349,835
10—15	1,450,170	5,375	10	19,822,355	20,194,425
15—25	2,614,970	16,135	15	16,927,390	17,288,710
25—35	2,222,620	24,505	25	11,723,585	12,052,635
35—45	1,669,670	32,640	35	7,302,850	7,582,890
45—55	1,102,130	34,325	45	3,996,150	4,210,910
55—65	621,470	33,810	55	1,826,215	1,972,325
65—75	264,820	27,185	65	617,085	695,575
75—85	59,210	10,790	75	114,630	138,750
85 and upwards	4,135	1,270	85	7,000	9,540

perhended if they are regarded from a geometrical point of view as perpendicular lines or “ordinates” erected upon a base line or “abscissa.” The process of interpolation by finite differences consists practically in drawing a curve of the n th degree through the upper extremities of $n+1$ of these ordinates. The ordinate erected at any intermediate point, *i.e.* at any intermediate age, measured from the abscissa to the curve, is the measure of the corresponding value of $2P-d$ or $2P+d$.

Therefore as we have the height of the ordinate given for age x and upwards, denoted by the symbol u_x , if we can get the measure of the corresponding ordinate for age $x+1$ and upwards, u_{x+1} , then $u_x - u_{x+1}$ will give the measure of $2P-d$, or $2P+d$ for age x to age $x+1$ and so from the fraction $\frac{2P-d}{2P+d}$ the value of p_x is easily deduced.

It is found best however to work *not* directly from the numbers but from the corresponding Logarithms, one chief reason for this being that there is thus possible to be obtained a rational continuation of the series below the point at which the actual data cease to be available, that is after the age 85.

The two right-hand columns in the above table are therefore translated into their corresponding logarithms as below.

$u_4 = 7.3730654$	$U_4 = 7.3804945$
$u_5 = 7.3608547$	$U_5 = 7.3682838$
$u_{10} = 7.2971553$	$U_{10} = 7.3052315$
$u_{15} = 7.2285900$	$U_{15} = 7.2377626$
$u_{25} = 7.0690604$	$U_{25} = 7.0810820$
$u_{35} = 6.8634924$	$U_{35} = 6.8798347$
$u_{45} = 6.6016418$	$U_{45} = 6.6243759$
$u_{55} = 6.2615519$	$U_{55} = 6.2949785$
$u_{65} = 5.7903451$	$U_{65} = 5.8423439$
$u_{75} = 5.0592983$	$U_{75} = 5.1422330$
$u_{85} = 3.8450980$	$U_{85} = 3.9795484$

$\log 2P - d$ at age x and upwards is denoted by the symbol u_x , and $\log 2P + d$ at age x and upwards by the symbol U_x .

At this point it must be noted that whereas we have used the symbol p_x to denote the chance of surviving from age x to age $x+1$, the values to be obtained by the method to be immediately described give the chance of survival as existing at the *exact age* x , or what may be taken practically as the chance of surviving from age $x - \frac{1}{2}$ to age $x + \frac{1}{2}$, and these values will hereafter be denoted by the symbol p'_x .

Now the general formula by which the *logarithms* of the p'_x values are to be obtained from the tables of u_x and U_x values given above is this:

$$\log p'_x = (u_x + \log b) - (U_x + \log B).$$

Thus for p'_5 the values of u_5 and U_5 can be at once written down in the formula.

From what has been previously said it will be understood that B is the coefficient of x in the expansion of the equation $u_x = A + Bx + Cx^2 + \dots$. For the sake of distinction the small letter b is used to denote the coefficient in the series $2P - d$, and the capital letter B the corresponding coefficient in the series $2P + d$. For the purposes of the equation for p'_x above given the values of b and B require a *separate calculation for each value of x* .

Two points may be here noted with regard to the above formula for p'_x . (1) The coefficient b (or B) is a negative quantity, but it may be left positive since $-\frac{b}{-B} = \frac{b}{B}$.

(2) Any *identical* multiples of b and B may be used in the equation since $\frac{xb}{xB} = \frac{b}{B}$.

The series from which p'_5 , p'_{10} , and p'_{15} have to be calculated is for b' , u_4 , u_5 , u_{10} , u_{15} , u_{25} , u_{35} and for B , the corresponding series commencing with U_4 .

The values of b and B can be thus expressed in terms of the series u_4 , u_5 , &c.

(1) In the equation for p'_5

$$b = \left[\left(\begin{array}{c} 883,190u_5 \\ + 152,768u_{10} \\ + 3,410u_{25} \end{array} \right) - \left(\begin{array}{c} 1,000,000u_4 \\ + 39,060u_{15} \\ + 308u_{35} \end{array} \right) \right] \div 1,432,200.$$

(2) In the equation for p'_{10}

$$b = \left[\left(\begin{array}{c} 1,250,000u_4 \\ + 343,728u_{10} \\ + 585,900u_{15} \\ + 2,772u_{35} \end{array} \right) - \left(\begin{array}{c} 2,148,300u_5 \\ + 34,100u_{25} \end{array} \right) \right] \div 5,728,800.$$

(3) In the equation for p'_{15}

$$b = \left[\begin{pmatrix} 1,575,420u_5 \\ +1,035,090u_{15} \\ +75,020u_{25} \end{pmatrix} - \begin{pmatrix} 1,000,000u_4 \\ +1,680,448u_{10} \\ +5,082u_{35} \end{pmatrix} \right] \div 4,296,600.$$

From what has been said above it will be obvious that only the *numerators* of the above fractions need be worked out.

In order to save labour the formulae given above for the numerators have been re-arranged as below so as to give the multiples of b indicated by the denominators.

(1) for p'_5

$$\left\{ (220-3) \times \begin{bmatrix} +4,070u_5 \\ +704u_{10} \end{bmatrix} \right\} + 3,410u_{25} - \begin{bmatrix} 1,000,000u_4 \\ +39,060u_{15} \\ +308u_{35} \end{bmatrix},$$

(2) for p'_{10}

$$\left\{ (220-3) \times \begin{bmatrix} +(11 \times 144)u_{10} \\ +(3,000-300)u_{15} \\ -(10,000-100)u_5 \end{bmatrix} \right\} + \begin{bmatrix} 1,250,000u_4 \\ +(3,080-308)u_{35} \end{bmatrix} - 34,100u_{25},$$

(3) for p'_{15}

$$\left\{ (220-3) \times \begin{bmatrix} \left(\begin{matrix} +(700-40)u_5 \\ -704u_{10} \end{matrix} \right) \times 11 \\ +(5,000-230)u_{15} \end{bmatrix} \right\} + (3,410 \times 22)u_{25} - \begin{bmatrix} 1,000,000u_4 \\ +(3,080+2,002)u_{35} \end{bmatrix}.$$

The labour may be still further diminished by subtracting *any one* of the given terms u_4 , u_5 , &c. *from all the terms* of the series, thus reducing the one subtracted from itself to zero, and then applying the formulae to the *new* series of values.

If the calculations have been first made with u_4 eliminated it is well to check the results by repeating the work with another term (say u_{15} or u_{35}) eliminated.

The working out of the above formula for p'_5 gives the following result:

$$\text{multiple of } b = -17617.5017318$$

$$\text{,, ,, } B = -17573.8315096.$$

$$\begin{aligned} \therefore \log p'_5 &= (7.3608547 + \log 17617.502) - (7.3682838 + \log 17573.832) \\ &= (7.3608547 + 4.2459444) - (7.3682838 + 4.2448665) \\ &= \bar{1}.9936488. \end{aligned}$$

$$\therefore p'_5 = .98548.$$

After having calculated the logs of p'_5 , p'_{10} , and p'_{15} the remaining values from $\log p'_{25}$ onwards are much more easily arrived at.

The formula given below in each case produces the value of $b \times -120$, and as before the similar multiples of b and B are used.

Formulae for multiples of b and B

$$\begin{aligned} \text{for } p'_{25} & 8(u_{15} - u_{35}) - (u_5 - u_{45}) \\ \text{for } p'_{35} & 8(u_{25} - u_{45}) - (u_{15} - u_{55}) \\ \text{for } p'_{45} & 8(u_{35} - u_{55}) - (u_{25} - u_{65}) \\ \text{for } p'_{55} & 8(u_{45} - u_{65}) - (u_{35} - u_{75}) \\ \text{for } p'_{65} & 8(u_{55} - u_{75}) - (u_{45} - u_{85}) \\ \text{for } p'_{75} & 8(u_{65} - u_{85}) - (u_{55} - u_{95}) \\ \text{for } p'_{85} & 8(u_{75} - u_{95}) - (u_{65} - u_{105}). \end{aligned}$$

(For B of course the corresponding U_x values are to be used.)

In order to obtain the values of u_{95} and u_{105} it is only necessary to difference the series u_{45} , u_{55} , u_{65} , u_{75} , u_{85} and to carry the differences down for two stages.

After having completed this stage of the work we shall have the following series of values as the foundation for the subsequent process of interpolation.

$$\begin{aligned} \log p'_5 &= \bar{1} \cdot 9936488 \\ \log p'_{10} &= \bar{1} \cdot 9983627 \\ \log p'_{15} &= \bar{1} \cdot 9980733 \\ \log p'_{25} &= \bar{1} \cdot 9965108 \\ \log p'_{35} &= \bar{1} \cdot 9933917 \\ \log p'_{45} &= \bar{1} \cdot 9893048 \\ \log p'_{55} &= \bar{1} \cdot 9823833 \\ \log p'_{65} &= \bar{1} \cdot 9663823 \\ \log p'_{75} &= \bar{1} \cdot 9359880 \\ \log p'_{85} &= \bar{1} \cdot 8842469. \end{aligned}$$

Now it would be quite possible to take these ten values and by one scheme of interpolation with nine orders of differences to obtain the required continuous series of intermediate values (in fact they have been worked through by the writer as far as p'_{35}). However the labour is too great, and the results so obtained are to be practically arrived at by an easier method, which consists in effecting interpolations in *several overlapping series* and then *joining* or "*welding*" these series with each other at certain parts, the final result being very nearly what would have been arrived at by the more laborious method.

The scheme which is recommended for adoption after the trial of others more elaborate as well as more simple, is to be represented as follows: the portion of each series used being included in brackets and the parts of series to be combined (by a method to be afterwards

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described) being indicated by over-lines and under-lines, *i.e.* the part *under-lined* is to be welded with the part of the series below it which is *over-lined*.

Series 1. 6 orders of differences

$$[p'_5, p'_{10}, p'_{15}, \overline{p'_{25}, p'_{35}}] p'_{45}, p'_{55}$$

Series 2. 5 orders of differences

$$p'_{15}, [\overline{p'_{25}, p'_{35}}, \overline{p'_{45}, p'_{55}}] p'_{65}$$

Series 3. 5 orders of differences

$$p'_{25}, p'_{35}, [\overline{p'_{45}, p'_{55}, p'_{65}}] p'_{75}$$

Series 4. 5 orders of differences

$$p'_{35}, p'_{45}, [\overline{p'_{55}, p'_{65}}, p'_{75}, p'_{85}, \dots]$$

It will thus be evident that the values of p'_6 to p'_{24} are derived from the first series alone, p'_{26} to p'_{34} by combining series 1 with series 2, p'_{36} to p'_{44} from series 2 alone, p'_{46} to p'_{54} by combining series 2 with series 3, p'_{56} to p'_{64} by combining series 3 with series 4, and from p'_{66} onwards from series 4 alone.

Series 1 is the one which entails most labour as the intervals between the terms are unequal.

Using the small letter δ instead of the capital Δ as the symbol representing the successive differences, and using the symbol u_0 to represent p'_5 , u_5 to represent p'_{10} , u_{10} to represent p'_{15} , u_{20} to represent p'_{25} , &c., &c., the following formulae will give the line of differences opposite to u_0 , viz. δu_0 , $\delta^2 u_0$, $\delta^3 u_0$, $\delta^4 u_0$, $\delta^5 u_0$ and $\delta^6 u_0$.

$$\delta^6 u_0 = \left[\begin{array}{c} -0\cdot001,024u_5 \\ -0\cdot000,18u_{40} \\ +0\cdot000,028u_{50} \end{array} \right] \div 21 + \left[\begin{array}{c} 0\cdot000,012u_0 \\ +0\cdot000,06u_{10} \\ +0\cdot000,024u_{30} \end{array} \right] - 0\cdot000,04u_{20}$$

$$\delta^5 u_0 = \left[\begin{array}{c} +0\cdot002,56u_5 \\ +0\cdot000,1u_{40} \end{array} \right] \div 7 - \left[\begin{array}{c} 0\cdot000,1u_0 \\ +0\cdot000,4u_{10} \\ +0\cdot000,08u_{30} \end{array} \right] + 0\cdot000,2u_{20} - 15\delta^6 u_0$$

$$\delta^4 u_0 = \left[\begin{array}{c} +0\cdot008u_0 \\ +0\cdot002,4u_{10} \\ +0\cdot000,16u_{30} \end{array} \right] - \left[\begin{array}{c} 0\cdot002,56u_5 \\ +0\cdot000,8u_{20} \end{array} \right] - 11\delta^5 u_0 - 64\cdot5\delta^6 u_0$$

$$\delta^3 u_0 = \left[\begin{array}{c} -0\cdot006u_0 \\ -0\cdot012u_{10} \end{array} \right] + \left[\begin{array}{c} 0\cdot016u_5 \\ +0\cdot002u_{20} \end{array} \right] - 7\cdot25\delta^4 u_0 - 28\delta^5 u_0 - 75\delta^6 u_0$$

$$\delta^2 u_0 = \left[\begin{array}{c} +0\cdot04u_0 \\ +0\cdot04u_{10} \end{array} \right] - 0\cdot08u_5 - 4\delta^3 u_0 - 8\delta^4 u_0 - 10\delta^5 u_0 - 8\cdot4\delta^6 u_0$$

$$\delta u_0 = -0\cdot2u_0 + 0\cdot2u_5 - 2\delta^2 u_0 - 2\delta^3 u_0 - \delta^4 u_0 - 0\cdot2\delta^5 u_0.$$

In order to verify the correctness of the values obtained the follow-

ing checking equation may be used which has been worked out from the general equation previously given.

$$u_{30} = u_0 + 50 \delta u_0 + 1,225 \delta^2 u_0 + 19,600 \delta^3 u_0 + 230,300 \delta^4 u_0 \\ + 2,118,760 \delta^5 u_0 + 15,890,700 \delta^6 u_0.$$

In applying the above formulae the labour may be diminished by subtracting u_0 from all the terms, thus reducing u_0 itself to zero, and then working from the *new* terms. Thus:

Original terms		New terms after subtracting u_0
$u_0 = p'_5 = \bar{1} \cdot 9936488 :$
$u_5 = p'_{10} = \bar{1} \cdot 9983627 :$
$u_{10} = p'_{15} = \bar{1} \cdot 9980733 :$
$u_{20} = p'_{25} = \bar{1} \cdot 9965108 :$
$u_{30} = p'_{35} = \bar{1} \cdot 9933917 :$
$u_{40} = p'_{45} = \bar{1} \cdot 9893048 :$
$u_{50} = p'_{55} = \bar{1} \cdot 9823833 :$

It will require extreme care with regard to the signs, a negative coefficient multiplied by a negative quantity giving a + result, &c.

The working out of the formulae gives the following results:

$$\begin{aligned} \delta^6 u_0 &= - : 6282552 \\ \delta^5 u_0 &= + 14 : 2743429 \\ \delta^4 u_0 &= - 154 : 2905086 \\ \delta^3 u_0 &= + 1046 : 5677300 \\ \delta^2 u_0 &= - 5090 : 7329360 \\ \delta u_0 &= + 17667 : 5660519 \end{aligned}$$

The sign : is conveniently used to denote the end of seven places of decimals.

If reference be made to the simple illustrative instance previously given no difficulty should be experienced in regard to the mode of procedure.

The differences are successively carried down by algebraical addition starting with $\bar{1} \cdot 9936488$ as u_0 , thus:

$$\begin{array}{rcl} & \bar{1} \cdot 9936488 & = u_0 = p'_5 \\ & + 17667 : 566052 & \\ \hline & \bar{1} \cdot 9954155 : 566052 & = u_1 = p'_6 \\ & + 12576 : 833116 & \\ \hline & \bar{1} \cdot 9966732 : 399168 & = u_2 = p'_7 \\ & + 8632 : 667910 & \\ \hline & \bar{1} \cdot 9975365 : 067078 & = u_3 = p'_8 \\ \hline \end{array}$$

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It is obvious of course that when each of the given values of p'_{10} , p'_{15} &c. is reached it will at once be seen whether any errors have been made, as the hypothetical curve being drawn should pass through the fixed points.

The formulae for the series (2), (3), and (4) are much simpler than those for series (1), and are the same for each series as the intervals between the given terms are equal.

In proceeding to deal with series (2), the first step is to set down and difference the given values of p'_{15} to p'_{65} , thus:

		Δ	Δ^2	Δ^3	Δ^4	Δ^5
p'_{15}	1̄9980733:	-15625:	-15566:	+5888:	-24556:	-19225:
p'_{25}	1̄9965108:	-31191:	-9678:	-18668:	-43781:	
p'_{35}	1̄9933917:	-40869:	-28346:	-62449:		
p'_{45}	1̄9893048:	-69215:	-90795:			
p'_{55}	1̄9823833:	-160010:				
p'_{65}	1̄9663823:					

The problem is to subdivide these differences represented by the symbol Δ^n into smaller differences δ^n corresponding to the tenth part of the interval.

The key to the solution of the problem is the equation

$$\Delta = \{(1 + \delta)^{10} - 1\}.$$

The working out of this equation to five orders of differences produces the following simple formulae:

$$\begin{aligned}\delta^5 &= \cdot00001 \Delta^5 \\ \delta^4 &= \cdot0001 \Delta^4 - 18\delta^5 \\ \delta^3 &= \cdot001 \Delta^3 - 13\cdot5\delta^4 - 96\cdot75\delta^5 \\ \delta^2 &= \cdot01 \Delta^2 - 9\delta^3 - 44\cdot25\delta^4 - 150\delta^5 \\ \delta &= \cdot1 \Delta - 4\cdot5\delta^2 - 12\delta^3 - 21\delta^4 - 25\cdot2\delta^5.\end{aligned}$$

It is essential to remember that these formulae must be applied to *a complete line of Δ^n values.*

In the instance now being dealt with the interpolation is required to begin at p'_{25} and it is only necessary to fill in the constant Δ^5 value -19225: at the blank space opposite to p'_{25} .

If as in series (3) and (4) the interpolation had had to begin at the *third* line the first blank space in the line would have had to be filled by the sum of -43781: and -19225:, and the last blank space as before by -19225:.

The following are the needful checking equations so as to carry the checking process *to the end of the series*, which is necessary to avoid error.

(a) For series (2)

$$u_{40} \text{ (i.e. } p'_{65}) = u_0 \text{ (i.e. } p'_{25}) + 40\delta + 780\delta^2 + 9,880\delta^3 + 91,390\delta^4 + 658,008\delta^5.$$

(b) For series (3) and (4)

$$u_{30} \text{ (i.e. } p'_{75}) = u_0 \text{ (i.e. } p'_{45}) + 30\delta + 435\delta^2 + 4,060\delta^3 + 27,405\delta^4 + 142,506\delta^5.$$

(In series (4) u_{30} is p'_{85} and u_0 is p'_{55} .)

When the values of δ^5 to δ are obtained the interpolation is proceeded with precisely as shown before.

On "Welding" or combining two series.

The next matter demanding explanation is the process to which allusion has been made, of combining overlapping portions of two adjacent series so that one curve shall pass into the other without any abrupt transition.

This method is one of the many improvements in Life-Table construction devised by Mr. A. C. Waters.

It is an adaptation of what is known as the "curve of cosines."

The figures in illustration given below show the process as applied to welding series (1) with series (2) at the interval between p'_{25} and p'_{35} , i.e. p'_{26} to p'_{34} .

It will be noted that the sum of each pair of multipliers = 1.

From series (1)		From series (2)		Combined values
$p'_{26} = 9963336 : 32$	$\times 0.976$	$+ 9962487 : 15$	$\times 0.024$	$= \bar{1} \cdot 9963316$
$p'_{27} = 9961318 : 92$	$\times 0.904$	$+ 9959728 : 09$	$\times 0.096$	$= \bar{1} \cdot 9961166$
$p'_{28} = 9959012 : 47$	$\times 0.794$	$+ 9956843 : 13$	$\times 0.206$	$= \bar{1} \cdot 9958565$
$p'_{29} = 9956385 : 47$	$\times 0.654$	$+ 9953843 : 67$	$\times 0.346$	$= \bar{1} \cdot 9955506$
$p'_{30} = 9953419 : 96$	$\times 0.500$	$+ 9950740 : 01$	$\times 0.500$	$= \bar{1} \cdot 9952080$
$p'_{31} = 9950112 : 59$	$\times 0.346$	$+ 9947541 : 14$	$\times 0.654$	$= \bar{1} \cdot 9948431$
$p'_{32} = 9946475 : 09$	$\times 0.206$	$+ 9944254 : 55$	$\times 0.794$	$= \bar{1} \cdot 9944712$
$p'_{33} = 9942534 : 08$	$\times 0.096$	$+ 9940886 : 06$	$\times 0.904$	$= \bar{1} \cdot 9941044$
$p'_{34} = 9938330 : 27$	$\times 0.024$	$+ 9937439 : 59$	$\times 0.976$	$= \bar{1} \cdot 9937461$

This process is facilitated by deducting the largest possible *common value* from each pair of p'_x values before multiplying, and then adding the two products to the common value. Thus

$$p'_{26} = 9960000 + (3336:32 \times 0.976) + (2487:15 \times 0.024) = \bar{1} \cdot 9963316.$$

(See Diagram A for a graphic illustration of the above figures, the height of the ordinates representing the numerical values of the logarithms.)

It is hoped that sufficient explanation and illustration have now

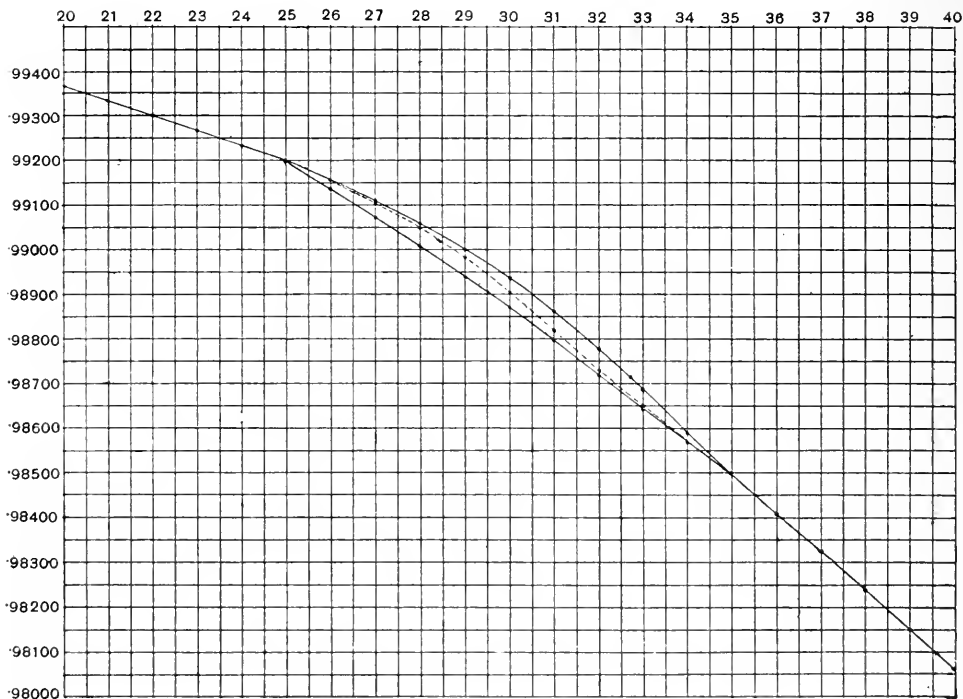
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been given to render it possible to calculate the required series of p'_x values from p'_5 right on to the end.

Precisely how far the last series will have to be carried can only be determined when the l_x column comes to be dealt with.

DIAGRAM A.

In this diagram the p'_x curve from p'_{20} to p'_{35} , derived from series 1 (see page 22), and the p'_x curve from p'_{25} to p'_{40} , derived from series 2, are shown separately by full lines, and the curve obtained by "welding" these two series, from p'_{25} to p'_{34} , is shown by an intermediate dotted line.



On the reduction of p'_x to p_x values.

However when these p'_x values are obtained and tabulated it must be remembered that they give the chance of living from age $x - \frac{1}{2}$ to $x + \frac{1}{2}$, and that before they can be made use of in the next stage of Life-Table construction, viz. the calculation of the number of survivors at each age out of a given number at birth, *i.e.* the l_x column, they

must be reduced to such values as shall represent the chance of living from age x to age $x + 1$.

The simplest mode of effecting this would be to take the geometrical mean of two consecutive p'_x values, or in other words the arithmetical mean of their logarithms, as the required intermediate value.

However, it is found that a more accurate and more evenly graduated curve is to be obtained by making each of the new p_x values to occupy the central point in the interval between the extremes of a series of four consecutive p'_x values.

The working out of the first value, viz. p_5 requires a special formula, so that the previously calculated p_4 value may be brought in. (It must be understood that p_x and p'_x are written down for the sake of brevity in the following formulae instead of $\log p_x$ and $\log p'_x$, and that the same remark applies to the preceding pages from page 22 onwards to this point, for in all these processes of interpolation and welding the logarithms are dealt with as if they were common numbers).

$$p_5 = \frac{-4p_4 + 15p'_5 + 10p'_6 - p'_7}{20}.$$

For the remaining p_x values only one formula is required which may be adequately represented by that for p_6 .

$$p_6 = \frac{10(p'_6 + p'_7) - (p'_5 + p'_6 + p'_7 + p'_8)}{16}.$$

The general rule simply is in order to deduce a p_x value from four p'_x values.

From ten times the sum of the two middle terms subtract the sum of all four terms and divide the remainder by 16, the result is the central term required.

These last interpolations can be very easily and quickly effected.

In order to avoid errors an important practical point is to difference the numerical values of the logs of the p_x values as one proceeds, as well as to plot them out to scale on paper ruled into squares.

(See diagram B for graphic illustration of the p_x curve from p_5 to p_{24} . This diagram also shows what extreme variations in this part of the p_x curve occur from different methods of interpolation, and that the method herein described at least gives a curve having some rational relation to the mean values of p_x deduced from the total population and death-numbers for the age-periods 5—10, 10—15, and 15—25.)

Calculation of the l_x column.

After having obtained the complete series of logs of p_x values the remaining work is of a comparatively simple nature.

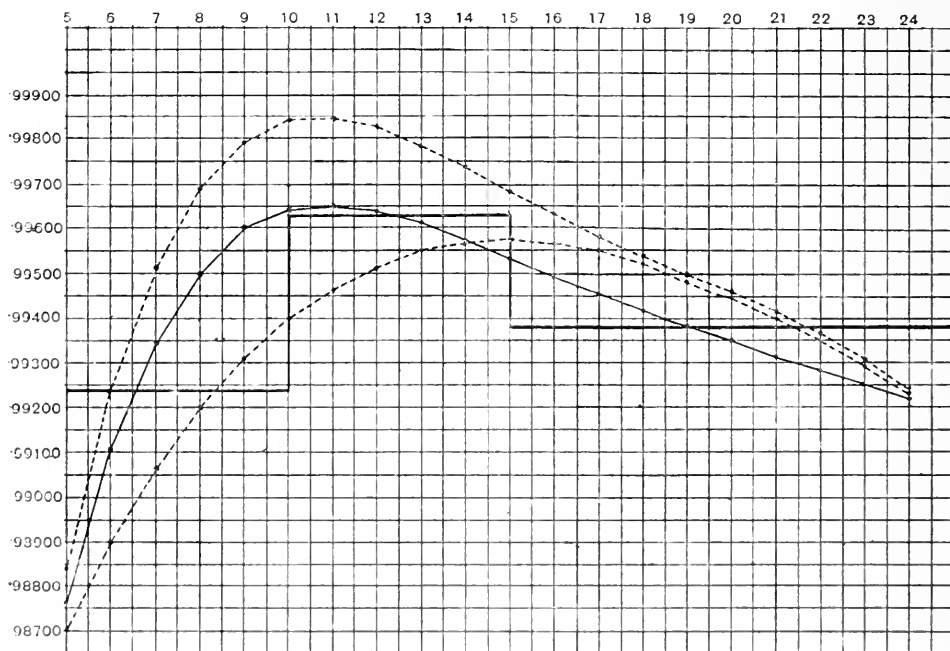
DIAGRAM B.

In this diagram are shown :

(1) by means of the full straight horizontal lines 5 to 10, 10 to 15, and 15 to 25, the mean p_x values derived from the years of life and the total deaths in ten years for each of the age-periods 5—10, 10—15, and 15—25, by the fraction $\frac{2P-d}{2P+d}$;

(2) by means of the full curved line, the p_x curve from p_5 to p_{24} , as derived by the final interpolation in p'_x values from series 1 (see page 27);

(3) by means of dotted curved lines, the p_x curves derived from the same data by two other methods of interpolation which have been employed in Life-Table construction, dealing with the data for the age-period 5—15 in *one* group instead of in the *two* groups 5—10 and 10—15.



The next step is to take some arbitrary number of individuals, assumed to set out together on the journey of life, as the l_0 number, i.e. the number "at birth," in actuarial terminology the "radix" of the Life-Table.

It does not matter what number is so taken, however, for the sake of being able to afterwards easily combine the results of the male and female sections of the Life-Table into one relating to *persons*, it is usual to divide up a hundred thousand or a million in proportion to the numbers of male and female births which have been registered during the decennium being dealt with.

The number l_0 which has been taken is next translated into the corresponding logarithm and then the logs of the p_x values are in succession added. Thus:

$$\begin{aligned}\log l_0 + \log p_{0 \text{ to } \frac{1}{2}} &= \log l_{\frac{1}{2}} = \log \text{survivors at age 6 months.} \\ \log l_{\frac{1}{2}} + \log p_{\frac{1}{2} \text{ to } 1} &= \log l_1 = \log \text{survivors at age 1 year.} \\ \log l_1 + \log p_1 &= \log l_2 = \log \text{survivors at age 2 years.} \\ &\quad \&c. \quad \&c.\end{aligned}$$

The process is continued until a negative "characteristic" is obtained, *i.e.* until the number of survivors falls below unity.

The logs of the l_x values are then to be translated into their corresponding numerical values.

In order to have a uniform standard of comparison for males and females as to the number of survivors at each age x it is usual to construct two other l_x columns, each starting with a hundred thousand or a million at birth.

This can be done

- (a) in precisely the same way as above described, or
- (b) assuming that we are taking a million as the l_0 value, by adding to each $\log l_x$ value already obtained ($\log 1,000,000 - \log l_0$ value previously taken). This will give the log of the new l_x value.

The d_x column, i.e. the successive numbers of those dying from age x to age $x+1$.

This is simply obtained by the formula $d_x = l_x - l_{x+1}$. It is obvious that $\Sigma d_x = l_x$, that is the sum of those dying from age x to the end of the Life-Table must equal those living at age x .

The P_x column.

This column, which represents the mean number living from age x to age $x+1$, for every year of life *except the first*, is taken as the *arithmetical mean* of l_x and l_{x+1} , *i.e.* $P_x = \frac{1}{2}(l_x + l_{x+1})$. (The arithmetical

mean is not only simpler but it can be shown to be *more accurate* than the geometrical mean, which has been used in some Life-Tables.)

The same number which expresses the *mean population* will also represent the *years of life lived* during the year x to $x+1$ by the number of survivors l_x entering upon that year of age; for, the years of life lived $= l_{x+1} + \frac{1}{2}d_x$,

$$= l_{x+1} + \frac{1}{2}(l_x - l_{x+1}) = \frac{1}{2}(l_x + l_{x+1}) = P_x.$$

The value of P_0 , that is the mean population for, or the years of life lived in, the *first* year of age is best calculated by the following simple formula which by an application of the Integral Calculus has been deduced by Mr A. C. Waters from the data, deaths at 0—3 mos., deaths at 3—6 mos., and deaths at 6—12 mos.

$$P_0 = l_1 + \left(\frac{d_{0 \text{ to } \frac{1}{2}} + 5d_{\frac{1}{2} \text{ to } 1}}{6} \right),$$

i.e. $= \frac{1}{6}$ deaths 0 to 6 mos. $+ \frac{5}{6}$ deaths 6 to 12 mos. $+ \text{survivors at age 1}$. As $l_{\frac{1}{2}}$ and l_1 will have been obtained, the values of $d_{0 \text{ to } \frac{1}{2}}$ and $d_{\frac{1}{2} \text{ to } 1}$ are to be deduced thus:

$$d_{0 \text{ to } \frac{1}{2}} = l_0 - l_{\frac{1}{2}}, \text{ and } d_{\frac{1}{2} \text{ to } 1} = l_{\frac{1}{2}} - l_1.$$

The Q_x column.

This is now to be constructed from the P_x column by successive additions beginning from below.

Reference to the previously explained double significance of the P_x numbers will make it apparent that each Q_x value will represent

(a) the years of life lived by l persons of exact age x during the year of age x to $x+1$ and *during all the years of age afterwards to the end of the Life-Table*, and

(b) the complete Life-Table population at age x and upwards.

The E_x column.

From the definition (a) of the Q_x column given above it is obvious that the expectation of life at age x , or in other words the mean after-lifetime of l individuals of exact age x will be equal to $\frac{Q_x}{l_x}$,

or

$$\log E_x = \log Q_x - \log l_x.$$

*Distribution of the total expectation of life at birth over the
different periods of life.*

It is of some importance in comparing different Life-Tables with each other to show how the total average expectation of life at birth (or E_0) is distributed over the different age-periods of life.

The periods of life from a practical point of view may be taken thus:

0—5, infancy
5—15, school age
15—65, working period of life
65— decline.

By dividing $Q_0 - Q_5$, $Q_5 - Q_{15}$, $Q_{15} - Q_{65}$, and Q_{65} respectively by l_0 the required values will be obtained, and, as will be obvious, the sum of the parts will equal the whole.

*How to deduce from the Q_x column the average expectation of life of
the individuals at all ages from x to $x + n$ comprised within the
age-groups x to $x + n$.*

By the methods of calculation which have been described it has been shown how from the data of the numbers living and dying at all intermediate ages within certain age-groups it is possible to calculate the mean after-lifetime of individuals at the *exact* age x .

However, for some of the most striking and important applications of a Life-Table it is necessary to re-translate these E_x values into those which represent the mean expectation of life of all the individuals comprised within the age-groups x to $x + n$, or, in other words, to make them applicable to a census population.

How this can be effected will be apparent from the following considerations.

(1) The future lifetime of P_x persons, *i.e.* of P persons living at all ages from x to $x + 1$ must be equal to $Q_x - \frac{1}{2}P_x$; for, on the assumption which is made in Life-Table construction, the average age of the P persons is $x + \frac{1}{2}$ at the middle of the year, therefore at the middle of the year they will on the average have each expended half of that year of life, and $\frac{1}{2}P_x$ must be deducted from the Q_x value.

(2) It is therefore obvious that their mean expectation of life individually must be equal to

$$\frac{Q_x - \frac{1}{2}P_x}{P_x} = \frac{Q_x}{P_x} - \frac{1}{2}.$$

(3) Similarly the future lifetime of $P_x + P_{x+1} + \dots + P_{x+n-1}$ persons, living at all ages between x and $x+n$, is represented by

$$(Q_x + Q_{x+1} + \dots + Q_{x+n-1}) - \frac{1}{2}(P_x + P_{x+1} + \dots + P_{x+n-1})$$

and their mean expectation of life =

$$\frac{(Q_x + Q_{x+1} + \dots + Q_{x+n-1}) - \frac{1}{2}}{(P_x + P_{x+1} + \dots + P_{x+n-1}) - \frac{1}{2}} = \frac{(Q_x + Q_{x+1} + \dots + Q_{x+n-1})}{(Q_x - Q_{x+n})} - \frac{1}{2}.$$

In actual practice of course the value of n is either 5 or 10, and from the Q_x column the average expectation of life can be readily calculated for the age-groups 0—5, 5—10, 10—15, 15—25 &c. &c.

For the purpose of this calculation it is sufficiently accurate to take as the mean expectation of life for the age-group 0—5

$$\frac{Q_0 + Q_1 + Q_2 + Q_3 + Q_4}{Q_0 - Q_5} - \frac{1}{2}.$$

II. ON THE USES OF A LIFE-TABLE FROM A PUBLIC HEALTH POINT OF VIEW.

Assuming that the laborious task which it has been the object of the preceding pages to explain has been completed, it remains to indicate in very brief outline the practical uses of the Life-Table.

That it is a most valuable statistical instrument for a community to possess will be apparent from the following considerations. By means of it the possibility exists of making exact comparisons

(1) With the Life-Table for the whole country for the same decennial period.

(2) With all other Local Life-Tables which may have been worked out for the same period.

(3) And, what is of very special importance, with any Life-Tables *for the same district* which may have been already calculated for previous decennial periods, thus giving the most exact measure possible for marking the effects of advance or retrogression in the conditions affecting health and life.

The special lines along which such comparisons may be with advantage made are the following.

(1) *The p_x values.*

These afford the means of testing the vitality of a community at each age or age-period.

They depend neither upon antecedent nor upon consequent conditions, but simply upon the "force of mortality" which has prevailed at each special age or age-period.

(2) *The l_x values.*

These depend upon the rates of mortality at *preceding* age-periods. Thus high death-rates during the earlier years of life diminish the number of survivors at later ages.

(3) *The E_x values.*

These are affected by the death-rates at all *following* age-periods.

Therefore p_x , l_x , and E_x are measures respectively of *present*, *past*, and *future*. (The death-rates of the decennial period on which the Life-Table is based are of course *simultaneous*, but they are assumed to exist in succession.) As has been already pointed out the distribution of the total expectation of Life at Birth over the successive age-periods of life may be readily arrived at and is an important point for comparison.

The expectation of Life at Birth having been obtained for males and females and the values being supposed to hold good for succeeding years until the next Life-Table is calculated, a balance of gain and loss can readily be struck for each year. Each Birth represents so many years of prospective lifetime, and the total prospective gain is readily calculated. Since the same number which expresses the estimated mean population for a year expresses also the years of life lived or expended in that year we have thus the loss to set against the gain.

Seeing that the mean expectation of Life has been obtained for the individuals of all ages within the usual age-groups, it is a simple matter, having given the estimated population living at the middle of a year, classified into the same age-groups¹, to calculate the total "Life-capital"

¹ It is of course necessary to assume that the estimated population for each year is composed of age and sex groups in the same proportions to the total as those ascertained at the preceding census.

of the community, and the division of this total by the whole population-number obviously gives the average Life-capital, or future life-time, of each individual of the population.

Finally if a calculation be made of the number of deaths which should have occurred in each age-group if the mean death-rates for the 10-yearly period of the Life-Table had continued unchanged, and if then a comparison be made between these numbers and the numbers of deaths which *actually have occurred* in the year being dealt with, it is a simple matter to strike the balance of gain or loss of Life-capital. It is obvious that lives lost or gained in the earlier age-groups have greater weight in the balance sheet than those at later ages.

III. ON CERTAIN MODIFICATIONS OF DR FARR'S "SHORT" METHOD OF LIFE-TABLE CONSTRUCTION by means of which, as regards Expectation of Life at quinquennial age-intervals, results can be obtained practically identical with those arrived at by the previously described "extended" method.

Going back to the point at which it had been described how to obtain the p_x values for the first five years of age, and from which proceeded the laborious path by which the remaining p_x values are to be one by one arrived at, it will be noted that attention has been drawn to the ease with which a mean value of p_x to $x+n$ can be worked out from the "years of life" and the total deaths in the ten years for each age-period by the fraction $\frac{2P-d}{2P+d}$.

The first step to be taken in the construction of a short Life-Table is to work out such a series of mean p_x values for the usual age-groups, viz. 5—10, 10—15, 15—25 and so on, ending with 85—.

To obtain p_{95-} it is best to put down the logs of p_{55-} , p_{65-} , p_{75-} and p_{85-} in a column, and then by differencing them and carrying down the differences for one stage, $\log p_{95-}$ is arrived at.

For the first few age-groups there is but little difference between the p_x values so found and the means of the separate yearly values obtained by the extended method, but afterwards the former become more and more in excess of the latter. See Table below.

Mean p_x values.

Age-periods	By extended method (a)	By short method (b)	Differences of (b) from (a)
5—10	·99247	·99241	—·00006
10—15	·99630	·99630	±·00000
15—25	·99380	·99384	+·00004
25—35	·98890	·98903	+·00013
35—45	·98037	·98064	+·00027
45—55	·96872	·96933	+·00051
55—65	·94514	·94704	+·00190
65—75	·89665	·90236	+·00571
75—85	·81742	·83299	+·01557
85—95	·70007	·72985	+·02978
95—	·58632	·59463	+·00831

Calculation of l_x values.

In using the above given mean p_x values it is simply necessary to take 5 times the mean value for a stage of 5 years, or 10 times the mean value for a stage of 10 years, etc.

Thus, commencing with

$$\begin{aligned}
 l_5 &= 34,467 \\
 \log l_5 + (\log p_{5-10} \times 5) &= \log l_{10} \\
 \log l_{10} + (\log p_{10-15} \times 5) &= \log l_{15} \\
 \log l_{15} + (\log p_{15-25} \times 10) &= \log l_{25} \\
 \text{\&c.} \qquad \qquad \qquad \text{\&c.}
 \end{aligned}$$

It is evident that by this method the l_x numbers will tend to differ more and more in the direction of excess as compared with those obtained by the extended method.

See Table given below.

Comparison of l_x values obtained by extended and short methods.

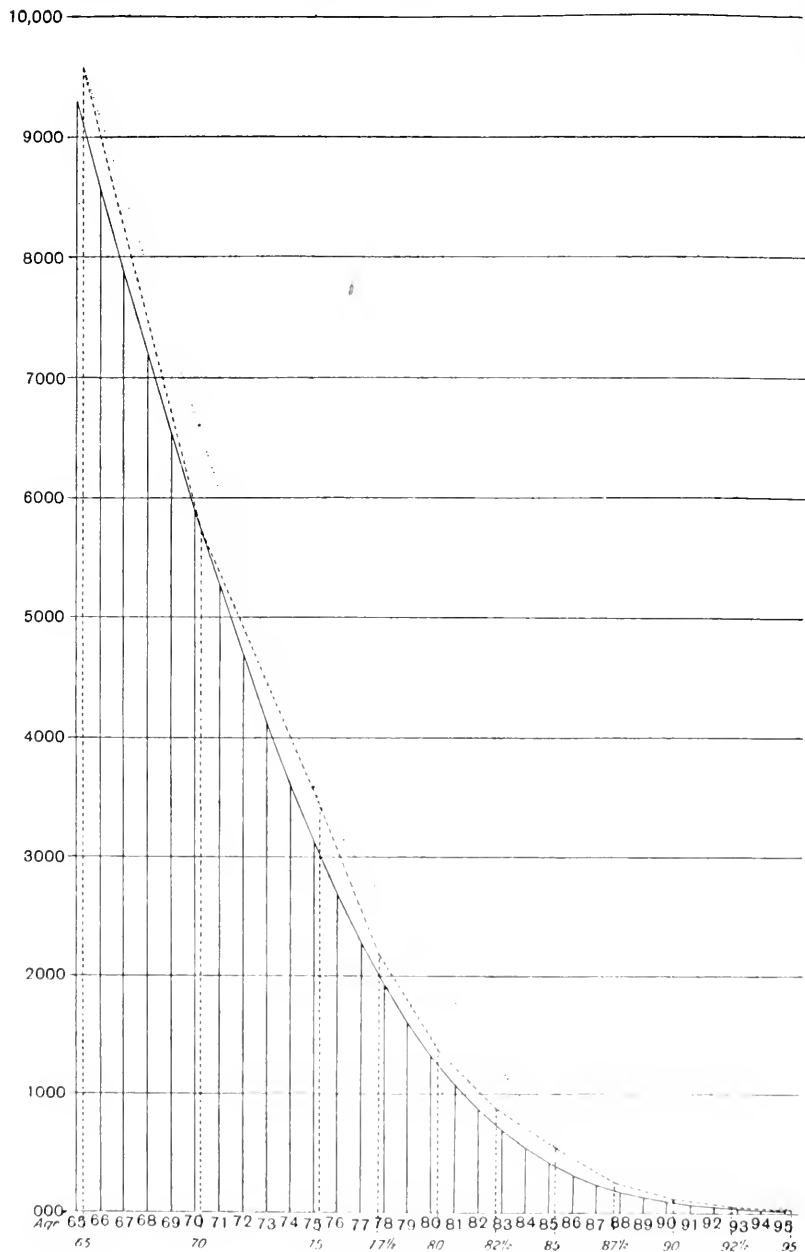
	By extended method (a)	By short method (b)	Differences of (b) from (a)
$l_{10} =$	33,190	33,178	—12
$l_{15} =$	32,580	32,569	—11
$l_{25} =$	30,615	30,617	+2
$l_{35} =$	27,381	27,420	+39
$l_{45} =$	22,458	22,551	+93
$l_{55} =$	16,344	16,516	+172
$l_{65} =$	9,296	9,585	+289
$l_{75} =$	3,123	3,431	+308
$l_{85} =$	416	552	+136
$l_{95} =$	12	24	+12

DIAGRAM C.

In diagram C the numbers shown at the base-line represent ages, and the vertical lines, or ordinates, represent the respective l_x values.

On the scale employed it has been impossible to show the construction further than age 95.

For other details of description see page 38.



Calculation of P_x values.

It will be remembered that in the extended method P_x was taken as $\frac{1}{2}(l_x + l_{x+1})$. This in geometrical construction is equivalent to joining the extremities of the ordinates l_x and l_{x+1} by a straight line. If measured with strict mathematical accuracy account would have to be taken of the *curve* passing through the extremities of the ordinates, but as the interval of one year is proportionally small the series of straight lines approximate very closely to the true curve. (See Diagram C.)

P_x was also found to be equal to $l_{x+1} + \frac{1}{2}d_x$ which is equivalent to considering that those dying between age x and age $x+1$ on the average live half through the interval.

Now if we come to apply these same assumptions to an interval of ten years,

$$P_{x \text{ to } x+10} = \frac{1}{2}(l_x + l_{x+10}) = l_{x+10} + \frac{1}{2}d_{x \text{ to } x+10},$$

and it is evident by reference to the diagram "C" that this geometrical construction must diverge more and more from the truth.

It will thus be evident why the E_x values obtained by Dr Farr's original short method diverged more and more from the values of an extended method in the direction of excess.

The general principle of the modification of Dr Farr's method which has been proposed by the writer is simply to take the 10-yearly intervals at subdivided stages. Thus each 10-yearly period from 15—25 to 65—75 inclusive is to be taken in *two* stages, the periods 75—85, and 85—95 are to be each taken in *four* stages, and from age 95 onwards *yearly* stages are to be used.

The series of l_x values required, therefore, will be as follows, having commenced the calculation with l_5 :

	l_{10}	l_{75}	$l_{87\frac{1}{2}}$	l_{95}
	l_{15}	$l_{77\frac{1}{2}}$	l_{90}	l_{97}
	l_{20}	l_{80}	$l_{92\frac{1}{2}}$	l_{98}
	l_{25}	$l_{82\frac{1}{2}}$	l_{95}	l_{99}
and so on to	l_{70}	l_{85}		and so on.

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The process of calculation is indicated as follows:

$$\begin{aligned}\log l_5 + (\log p_{5-10} \times 5) &= \log l_{10} \\ \log l_{10} + (\log p_{10-15} \times 5) &= \log l_{15} \\ \log l_{15} + (\log p_{15-25} \times 5) &= \log l_{20} \\ \log l_{20} + (\log p_{15-25} \times 5) &= \log l_{25} \\ \log l_{75} + (\log p_{75-85} \times 2\frac{1}{2}) &= \log l_{77\frac{1}{2}} \\ \log l_{77\frac{1}{2}} + (\log p_{75-85} \times 2\frac{1}{2}) &= \log l_{80}\end{aligned}$$

and so on.

The P_x values may be then easily arrived at, but it must be borne in mind that when the intervals are more than one year the *mean number living from age x to age $x + n$* will not express the *years of life lived from age x to $x + n$* , which must be obtained by multiplying P_x by n , and therefore before we can calculate the Q_x column there must be an intermediate column which may be called Y_x . Thus:

$$\begin{aligned}P_{5-10} &= \frac{1}{2}(l_5 + l_{10}) & Y_{5-10} &= P_{5-10} \times 5 \\ P_{10-15} &= \frac{1}{2}(l_{10} + l_{15}) & Y_{10-15} &= P_{10-15} \times 5 \\ P_{15-20} &= \frac{1}{2}(l_{25} + l_{20}) & Y_{15-20} &= P_{15-20} \times 5\end{aligned}$$

and so on to P_{70-75} .

$$\begin{aligned}P_{75-77\frac{1}{2}} &= \frac{1}{2}(l_{75} + l_{77\frac{1}{2}}) & Y_{75-77\frac{1}{2}} &= P_{75-77\frac{1}{2}} \times 2\frac{1}{2} \\ P_{77\frac{1}{2}-80} &= \frac{1}{2}(l_{77\frac{1}{2}} + l_{80}) & Y_{77\frac{1}{2}-80} &= P_{77\frac{1}{2}-80} \times 2\frac{1}{2}\end{aligned}$$

and so on to P_{90-95} .

$$\begin{aligned}P_{95} &= \frac{1}{2}(l_{95} + l_{96}) = Y_{95} \\ P_{96} &= \frac{1}{2}(l_{96} + l_{97}) = Y_{96}\end{aligned}$$

and so on.

Let the P_x and Y_x values be tabulated opposite to the corresponding l_x values. The Q_x column may now be constructed from the Y_x column by successive additions beginning from below, and then the E_x values may be readily calculated as before by the formula $E_x = \frac{Q_x}{l_x}$. These values, however, are only to be worked out for ages 5, 10, 15, 25, 35, 45, 55, 65, 75, 85 and 95.

In the diagram "C" the geometrical construction of the l_x , P_x , Y_x and Q_x columns is shown from age 65 onwards.

(a) By the extended method in full black lines.

(b) By the modified short method in interrupted black lines. (It must be supposed that this diagram has been superimposed upon the preceding and then moved a little to the right so as to show the construction.)

(c) The dotted black lines joining the ordinates at 10-yearly intervals indicate the construction of Dr Farr's original method.

Interpolation of intermediate quinquennial E_x values.

The method above described is only intended and adapted for obtaining E_x values at *decennial* intervals from E_{15} onwards.

However, by using very simple formulae like those which have already been described with relation to the interpolation of p_x values in a series of p'_x values, it is readily possible to obtain the values of E_{20} , E_{30} , &c., &c.

From E_{30} to E_{90} the following formula is applicable (with obvious successive changes in the suffixes):

$$E_{30} = \frac{10(E_{25} + E_{35}) - (E_{15} + E_{25} + E_{35} + E_{45})}{16},$$

E_{20} and E_{90} require special formulae, thus :

$$E_{20} = \frac{E_{15} + E_{35}}{4} + 1\frac{1}{2}E_{25} - E_{30},$$

$$E_{90} = \frac{E_{75} + E_{95}}{4} + 1\frac{1}{2}E_{85} - E_{80}.$$

Comparison of results obtained by the above described short method with those to be arrived at by an extended method.

In order to show the value of the modified short method its results as applied to the data for England and Wales 1881—90 are given below, and these are contrasted with those of an extended method. The values of E_0 , E_5 , E_{10} and E_{15} shown for the extended method have been recalculated by a method to some extent similar to what has been already described. The other values from E_{20} onwards have been taken from the official Life-Table.

Comparative Table. Section A.

Comparison of E_x values, *i.e.* mean expectation of life, or mean after-lifetime at exact age x obtained by (a) extended method, and (b) a modified short method.

N.B. The comparison is based on a new set of p_x values from p_5 to p_{24} which have been worked out for (a).

England and Wales, 1881—90.

Age x	Males			Females		
	(a)	(b)	Differences of (b) from (a)	(a)	(b)	Differences of (b) from (a)
0	43·28	43·32	+0·04	46·66	46·67	+0·01
5	52·24	52·30	+0·06	54·26	54·27	+0·01
10	48·59	48·65	+0·06	50·64	50·65	+0·01
15	44·28	44·33	+0·05	46·40	46·40	$\pm 0·00$
20	40·27	40·28	+0·01	42·42	42·40	-0·02
25	36·28	36·34	+0·06	38·50	38·51	+0·01
30	32·52	32·53	+0·01	34·76	34·74	-0·02
35	28·91	28·87	-0·04	31·16	31·08	-0·08
40	25·42	25·38	-0·04	27·60	27·51	-0·09
45	22·06	22·04	-0·02	24·05	24·01	-0·04
50	18·82	18·79	-0·03	20·56	20·50	-0·06
55	15·74	15·71	-0·03	17·23	17·12	-0·11
60	12·88	12·84	-0·04	14·10	14·00	-0·10
65	10·31	10·24	-0·07	11·26	11·17	-0·09
70	8·04	7·98	-0·06	8·77	8·71	-0·06
75	6·10	6·06	-0·04	6·68	6·62	-0·06
80	4·52	4·53	+0·01	5·00	4·98	-0·02
85	3·29	3·32	+0·03	3·71	3·69	-0·02
90	2·37	2·40	+0·03	2·75	2·75	$\pm 0·00$
95	1·72	1·72	$\pm 0·00$	2·05	1·97	-0·08

Comparative Table. Section B.

The next table shows a similar comparison with regard to the distribution of the total expectation of life *at Birth* over the age-periods indicated.

Age-period	Males			Females		
	(a)	(b)	Differences of (b) from (a)	(a)	(b)	Differences of (b) from (a)
0—5	4·02	4·02	$\pm 0·00$	4·16	4·16	$\pm 0·00$
5—15	7·33	7·34	+0·01	7·63	7·65	+0·02
15—25	7·05	7·03	-0·02	7·35	7·33	-0·02
25—35	6·62	6·61	-0·01	6·90	6·89	-0·01
35—45	5·97	5·97	$\pm 0·00$	6·30	6·30	$\pm 0·00$
45—55	5·10	5·10	$\pm 0·00$	5·55	5·54	-0·01
55—65	3·91	3·91	$\pm 0·00$	4·49	4·48	-0·01
65 and upwards	3·28	3·34	+0·06	4·28	4·32	+0·04
Totals	43·28	43·32	+0·04	46·66	46·67	+0·01

It is thus evident that as regards the two applications of a Life-Table indicated in the preceding two tables the short method gives results so close to those of the extended method as to be practically identical with them in most cases.

Until recently the writer had thought that it was not possible without the aid of an extended Life-Table to arrive at those striking results which are connected with the term "Life-capital." However, it has been found that by certain simple methods to be afterwards described the results are to be obtained set down in the following Table.

Comparative Table. Section C.

Mean expectation of Life of the individuals at all ages from x to $x + n$ comprised within the age-groups indicated.

Age-group x to $x+n$	Males		Differences of (b) from (a)	Females		Differences of (b) from (a)
	(a)	(b)		(a)	(b)	
0—5	51·76	51·76	$\pm 0\cdot00$	54·07	54·07	$\pm 0\cdot00$
5—10	50·57	50·58	$+ 0\cdot01$	52·60	52·56	$- 0\cdot04$
10—15	46·43	46·49	$+ 0\cdot06$	48·52	48·53	$+ 0\cdot01$
15—25	40·27	40·33	$+ 0\cdot06$	42·44	42·46	$+ 0\cdot02$
25—35	32·60	32·61	$+ 0\cdot01$	34·83	34·82	$- 0\cdot01$
35—45	25·51	25·48	$- 0\cdot03$	27·66	27·65	$- 0\cdot01$
45—55	18·95	18·93	$- 0\cdot02$	20·67	20·63	$- 0\cdot04$
55—65	13·09	13·06	$- 0\cdot03$	14·30	14·24	$- 0\cdot06$
65—75	8·35	8·34	$- 0\cdot01$	9·08	9·06	$- 0\cdot02$
75—85	4·94	4·94	$\pm 0\cdot00$	5·41	5·41	$\pm 0\cdot00$
85—95	2·80	2·78	$- 0\cdot02$	3·16	3·11	$- 0\cdot05$
95—	1·57	1·62	$+ 0\cdot05$	1·86	1·87	$+ 0\cdot01$

*Methods of arriving at above results, i.e. those of
section C, column (b).*

(1) It is presumed that a short Life-Table has been constructed by the method previously described, and that therefore the values of E_x have been obtained which are set down in "A" the first of the three sections of the comparative table of which "C" is the last.

(2) In the construction of this short Life-Table P_x and Q_x values have been obtained and set down in columns opposite to the l_x values as already described.

(3) The value of E_{0-5} is of course the same for (b) as for (a), as it is calculated alike for both methods (see description already given).

For E_{5-10} take the arithmetical mean of E_5 and E_{10} in Section A, column (b), and add 0·1.

For E_{10-15} and E_{15-25} take the arithmetical means respectively of E_{10} and E_{15} and E_{15} and E_{25} in Section A, column (b).

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For E_{25-35} , find the value of $\frac{Q_{25} + Q_{30}}{P_{25} + P_{30}} - 2\frac{1}{2}$ and take the arithmetical mean of this and E_{30} in Section A, column (b).

Similarly for E_{35-45} to E_{65-75} .

For E_{75-85} , find the value of $\frac{Q_{75} + Q_{77\frac{1}{2}} + Q_{80} + Q_{82\frac{1}{2}}}{P_{75} + P_{77\frac{1}{2}} + P_{80} + P_{82\frac{1}{2}}} - 1\frac{1}{4}$ and take the arithmetical mean of this and of E_{80} in Section A, column (b).

Similarly for E_{85-95} .

For E_{95-} take the E_{95} of Section A, column (b), and *subtract* 0.1.

CONCLUSION.

The question may now well arise in the minds of those who are contemplating the construction of merely local Life-Tables as to whether it is worth while to embark on the undertaking of working by the extended method.

The choice must be left to "personal equation." If anyone should try both methods he will at least appreciate how much labour is saved by the modified short method.

But for necessary limitation of space the difficult subject which it has been endeavoured to elucidate might have been dealt with more fully. The writer can only express the hope in conclusion that what has been given may be sufficient to render this paper a practical guide to Life-Table construction.

NOTES ON AN OUTBREAK OF CATTLE-PLAGUE IN SHANGHAI, AND ITS LIMITATION BY THE GALL IMMUNISATION OF KOCH.

By ARTHUR STANLEY, M.D., B.S. Lond., D.P.H.,

Health Officer of Shanghai.

THAT Cattle-plague (Rinderpest) is one of the most fatal and contagious maladies of cattle needs no further demonstration. The disease appears to be widely distributed in China, and is endemic in the hinterlands of Shanghai, Chinkiang, Hongkong, Peking, Tientsin, and Chefoo.

After an incubation period of about two days the onset of the disease is first manifested by increased temperature only: later loss of appetite appears, followed by constitutional signs of severe illness, drooping head, distressed look, standing coat, discharges from eye, nose and mouth, and diarrhoea, frequently bloody. Death usually takes place about the seventh day after the first rise of temperature. At autopsy the diagnosis can be confirmed by finding the pyloric region of the intestinal tract deeply congested, with patches of extravasation, erosion, and even necrosed yellowish areas which may have formed sanguinolent ulcers.

It has been completely established that the actual cause of the disease, the toxin, and the antitoxin, occur in the blood, and that immunity can be produced in cattle broadly as follows:

- | | |
|--|--------------------|
| 1. By injection under the skin of gall from an animal dead of cattle-plague (Koch). | } Passive immunity |
| 2. By injection of blood of an animal which has had cattle-plague. | |
| 3. By injection of blood of an animal which has had cattle-plague, together with blood of an animal which has cattle-plague (simultaneous method of Kolle and Turner). | } Active immunity |
| 4. By a previous attack of the disease. | |

Inasmuch as the production of active and lasting immunity (salting) by the simultaneous method of Kolle and Turner (antitoxic serum and virulent blood) causes a certain proportion of deaths, many animals to be sick, and the secretion of milk to be diminished, it would not be readily accepted voluntarily by the Chinese dairy-keepers. Moreover the local outbreaks occur at such irregular intervals (being absent often for several years) that a supply of antitoxic serum would be difficult to ensure at the required time. It is only necessary for the Sanitary Authority to protect the licensed dairies, for the dairy cattle are comparatively valuable Australian and half-bred milk cattle, difficult to replace locally, while the cattle used for slaughter are derived from immense and inexhaustible agricultural districts along the Grand Canal, where cattle-plague is endemic. It has appeared, therefore, that the gall immunisation method of Koch was most applicable to these isolated and limited herds of cattle in the Shanghai dairies and fulfilled the essential requirement, namely, rapid limitation of the disease when introduced. As strict sanitary inspection is exercised by the Health Department of the Shanghai Municipal Council over the dairies it is comparatively easy to apply the method. Moreover, on the occurrence of an epidemic means are immediately at hand of procuring the prophylactic gall, and the technique is simple and easily applicable to a large number of animals. The infected dairy can be first treated, then those adjacent, and finally those more remote. In this way the animals not yet infected are protected, and an epizootic prevented.

The origin of the present outbreak was as follows: A large herd of cattle infected with cattle-plague was brought to Shanghai from the hinterland (Tanyang district around the Grand Canal) for export to the allied troops in the north of China. The disease spread to an adjacent dairy, most of the cattle dying. On this dairy becoming infected a police cordon was established round it to prevent ingress and egress of cattle and ingress of persons unconnected with the dairy; while the outside infected herd was removed to an isolated part of the settlement. Having been previously convinced of the futility of police cordons in the prevention of cattle-plague, I was not surprised to find, within a short time, that the disease had spread, by the meeting together of cattle-coolies at a common tea-house, to three other dairies at a distance of a quarter, a half, and two miles from the original source of infection. As the animals are not as a rule taken away from the immediate vicinity of the dairy, there being no grazing fields, and as neither fodder

nor dung is taken from one dairy to another, it is practically certain the infection was carried by the dairy-coolies.

Immediately on this second series of dairies becoming infected it was resolved to apply the gall immunisation method of Koch, as being the means at hand. With this object an animal dying of the disease was opened up by a transverse incision across the right side of the upper part of the abdomen. The diagnosis was confirmed by finding the pyloric region both of the stomach and bowel congested and eroded. The gall-bladder was seized around the neck in the hollow of the hand and excised by cutting into the liver substance. The cut surface of the bile-duct was sterilised by washing with alcohol, and about 1500 c.c. of gall was slowly jetted into a sterile wide-mouthed bottle. The gall was clear and green, and 10 c.c. of it were injected into the dewlap of each of the 20 remaining cattle in the dairy. The rest of the gall was mixed with an equal part of glycerin and kept in an ice-chest till again required, the dose used of the glycerin-gall being 20 c.c., equal to 10 c.c. of pure gall. This was employed in the remaining infected dairies. Of three dead animals opened for the furnishing of gall two yielded clean green gall, while in the third the gall was thick and red, and was rejected. This gall mixed with an equal quantity of glycerin remains up to the present time (two months after collection) clear and green, and is sterile when inoculated on agar.

The injection of the gall into the dewlap caused slight local swelling and tenderness but no constitutional symptoms and no alteration in the milk supply, an important matter in a dairy. In all, 68 cattle were injected with cattle-plague gall. Of these 17 were among isolated uninfected herds, the remaining 51 belonged to infected herds; and among the latter 11 died of cattle-plague subsequent to the injection; namely, 4 on the fifth day, 2 on the seventh day, 1 on the eleventh day, 2 on the twelfth day, 1 on the fourteenth day, and 1 on the twentieth day after injection with gall. Taking eight days as the maximum incubation period of the disease and seven days as the usual period after onset of the disease that death may take place, it is clear that an animal dying of cattle-plague before the fifteenth day may have contracted the disease previous to the injection of the gall and hence may be eliminated in estimating the immunity gained. One animal, therefore, out of the 51 injected with gall and exposed to infection, with certainty contracted the disease after the injection, namely, that one which died twenty-one days after injection. Considering the usual

excessive mortality during an outbreak of this disease the result may almost be compared to the success of vaccination against small-pox.

Three young bullocks, each having received 20 c.c. of cattle-plague gall, were purposely exposed to severe infection. They remained well, while unprotected animals around them died of the disease.

In China generally the division of the land into small holdings, each owner keeping one, two, or at most three cattle, chiefly for the purpose of ploughing, is a sufficient safeguard, as regards isolation, against the disease, though endemic, causing great loss. There are in China no large herds except those acquired from the small owners by the cattle-dealers, who rapidly transfer the animals to the butchers for the non-Chinese consumers. It is, however, in the great centres where population, Chinese and non-Chinese, is aggregated, and herds of cattle for slaughter and dairy cattle come in touch, that cattle-plague becomes a source of great loss. And here its effect is chiefly felt among the permanent herds, *i.e.* among the dairy cattle only. The Chinese do not drink milk. It, therefore, becomes necessary to render these dairy cattle immune in non-Chinese communities.

To produce under these circumstances a permanent immunity (salting) by Kolle and Turner's method is a matter requiring considerable preparation and means at hand. This method is, therefore, difficult to apply in an emergency, *i.e.* in isolated places among small herds whose units are constantly changing. It has, therefore, appeared that the attainment of a permanent immunity of a proportion of the herd (a method which always causes the loss of a certain proportion of the herd in its production) may be sacrificed and an outbreak of the disease met by the means in hand, cattle-plague gall, which produces an immunity lasting for some four months (causing no loss of animals nor even temporary illness in its attainment) and which can be easily re-applied.

CONCLUSION.

The method of immunisation by gall being inexpensive and easily applicable, when once a case of cattle-plague has furnished the gall, may be considered perhaps the best for meeting future outbreaks of the disease in isolated places like Shanghai among small herds of dairy cattle.

THE GEOGRAPHICAL DISTRIBUTION OF ANOPHELES AND MALARIAL FEVER IN UPPER PALESTINE.

By JOHN CROPPER, M.A., M.B., B.C.

Late of Acca, Palestine.

[Thesis for the Degree of M.D., University of Cambridge.]

THE observations here recorded were made, for the most part, during a journey through Upper Palestine and Lower Syria, in June and July, 1901. The country traversed is indicated upon the accompanying map. Its character is so well known as not to need detailed description, being for the most part very hilly and rocky or level plain.

The chief rainfall occurs from November to April; December, January, and February being the wettest months. The annual rainfall amounts to between 20 and 30 inches, being heaviest on the coast. From May to November the weather is dry and fine, rain very seldom falling; when it does, however, its rapid absorption by the parched ground precludes the formation of pools.

Malarial fevers are most prevalent from June to November, reaching their maximum in October.

Last year (1900) during June and July, though constantly on the look-out for mosquitoes of all kinds, I failed to come across *Anopheles* at any place visited, except at Shibáh, high up (at least 5000 ft.) on the western slopes of Hermon. This was no doubt due to our avoidance of places where we ran risk of malarial infection: our only visits to marshy spots being made in the daytime. We then ascertained that at many of the most elevated villages malaria is almost, if not quite, unknown, and we were hardly ever asked for quinine. In view of this fact we informed ourselves among persons who knew the country well, regarding the most malarious localities. These naturally were for the greater part low-lying or marshy, but one of the most

malarious villages visited, viz. Banias (Cæsarea Philippi), is over 1000 ft. above sea-level, and *Anopheles* was found at Shibáh, quoted above, where the springs of water are icy cold, and the nights often of the same character, even in midsummer.

I have assumed no place to be malarious without either the opinion of the medical men of the place, or evidence obtained on the spot, chiefly in the direction of enlarged spleens. Without this precaution one can easily be deceived as to the character of many places.

Whilst travelling a month in malarious localities I used a tent for protection during the day, sleeping always at night in the open with no other protection than that afforded by mosquito netting. I might add that in order the better to convince those living in the country, of the part which it does *not* play in the etiology of malaria, I drank the water at every place visited where there seemed to be no danger of contamination from surface drainage. My attendants were a cook and a man to look after the horses, both of whom after a little grumbling consented to sleep under mosquito netting. None of us took quinine and we have all escaped malarial infection. A small shallow net of muslin stretched on a wire ring which could be mounted on a stick was used for catching *Anopheles* larvae. The imagines were as a rule caught in thick test-tubes, which I found most convenient: four or five specimens can be secured in each by inserting a small ball of paper after each insect is caught. Three or four tubes thus suffice for 15 to 20 mosquitoes, and the tubes can easily be carried in a box in the pocket. Imagines were rarely caught by means of a net, the latter being found to be clumsy and far less efficient. A torn net hung up loosely at night proved to be a most effective trap, and mosquitoes thus caught were in excellent condition. We thus, on one occasion, caught over 230 imagines in a single night. Test-tubes also served as receptacles for larvae, which like the imagines were quickly killed when carried in larger glass jars: especially was this the case when driving in a carriage over rough roads.

Specimens of *Culicidae* from each locality were secured and pinned in the usual way, and have since my return been examined very kindly at the British Museum by Mr F. V. Theobald, who considers two of the *Culices* to be hitherto undescribed. I have also to acknowledge the help given me by Lieut.-Col. Giles, I.M.S., in my entomological outfit.

I should here like to call attention to an excellent substitute for fine entomological pins, should these run short, in the shape of the spines of the *Centaurea calcitrapa*, var. *pallescens*, the thistle-like weed

found so abundantly in hot countries. Specimens mounted on these have travelled as well as those on pins, and are of course not liable to veridigris.

The accompanying map shews the route traversed, the more distinctly malarial foci being shaded. The large black dots indicate the presence of *Anopheles*, mostly *A. maculipennis* Meigen or *A. superpictus* Grassi: the former most common as imagines in houses, the latter as larvae: so much was this the case that almost all the mosquitoes hatched from larvae proved to be of this latter species.

Four species of *Anopheles* in all have been identified by Mr Theobald, *A. annularis*, sub-species *pseudopictus* Grassi so common in Italy, being enormously abundant in the papyrus marshes of the Upper Jordan valley, but not at any great distance from the marsh proper. The three species above mentioned are for the most part European, but an African species described by Mr Theobald as *A. pharoensis* (first found at Zomba in East Africa, near the Zambesi) proved to be the most interesting of all. Only five specimens of a variety of this very distinct and well-marked species were caught by us in Palestine.

I quite failed to find *Anopheles* at several points where malaria occurs sporadically, *i.e.* at Sidon and Acca and some villages on the edge of the plain, situated but slightly above sea-level. Near all of these places intensely malarial foci exist where *Anopheles* is easily found.

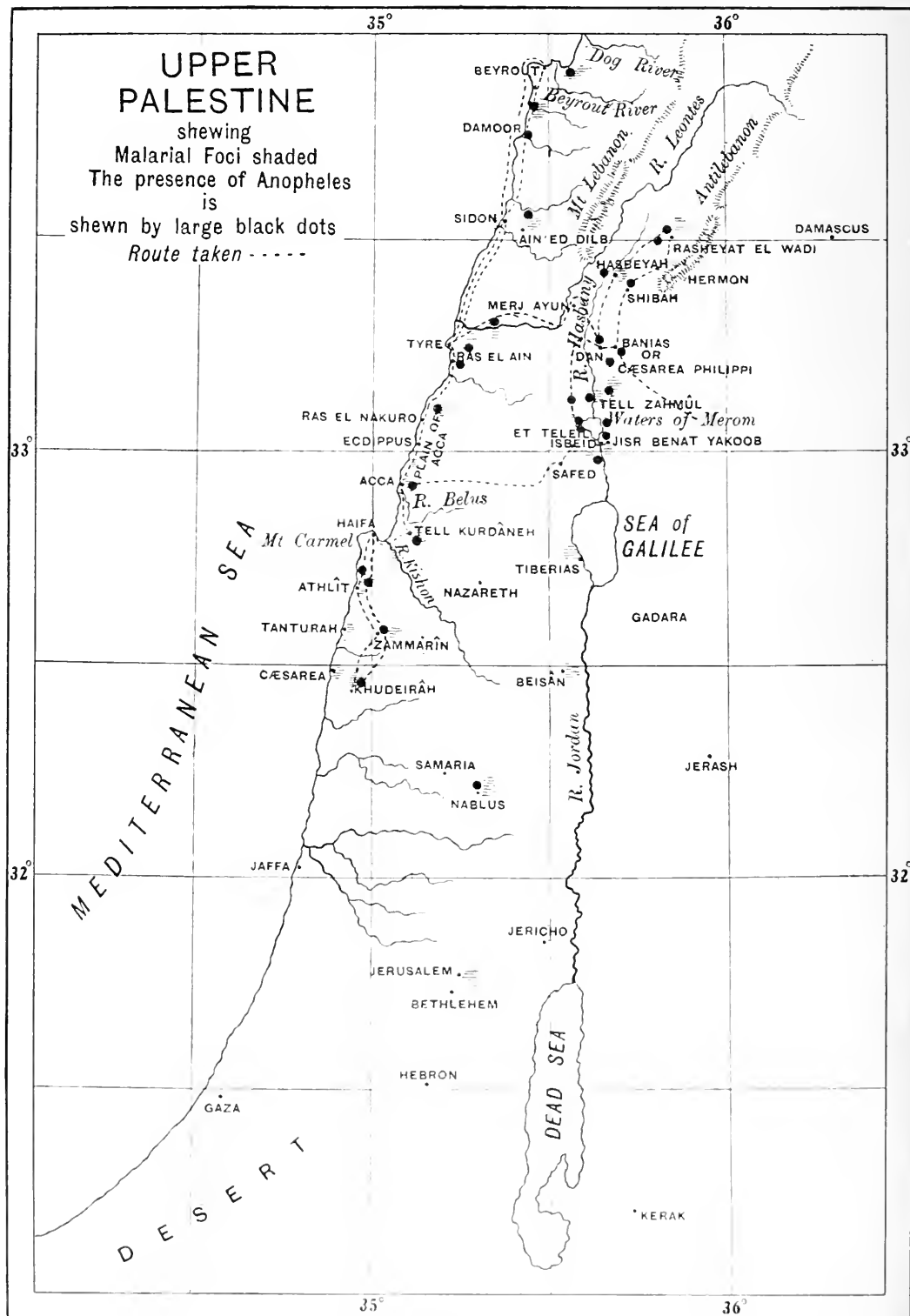
A few details regarding the places marked on the map will illustrate what I have said above: these being considered in the order in which they were visited.

Beyrout. This is for the most part healthy, being well drained and hilly, the soil dry and sandy. *A. superpictus* larvae were abundant on June 9th in a runnel of water containing *Spirogyra*, near the Beyrout river, though the latter was free from larvae. This was at the end of a fruitless search of some hours in company with Prof. E. Day of the American College in Beyrout, whose help I gladly here acknowledge. The unhealthiness of this district is indicated by the name Nahr el Môt, or River of Death, given to a stream not far distant. We had not time to examine other similar places nearer the Dog River. None of the open stone cisterns used for irrigation contained other than *Culex* larvae.

Damoor. Passing down the coast *Anopheles* larvae were found (in July) at the mouths of the rivers Nahr el Aweli and Nahr Damoor, in sandy channels, within reach of the waves at any rate in winter. These

UPPER PALESTINE

shewing
Malarial Foci shaded
The presence of Anopheles
is
shewn by large black dots
Route taken - - - -



channels contained *Spirogyra*, as in every other locality where *Anopheles* was observed.

Sidon. Here I failed to find *Anopheles* in the town, but *Culex pipiens* and *C. fuscatus* were, as everywhere on the coast, abundant. At Ain ed Dilb, a narrow valley or *wadi* in the chalk, I found *A. superpictus* larvae in great plenty, the imagines being caught on rocks on the edge of the stream at sunset. On our return, six weeks later, *A. superpictus* was still more abundant, being found together with *C. mimeticus*, which mimics this fly in its markings.

An outbreak of fever occurred amongst the workmen employed last year in building an American Orphanage near Ain ed Dilb, and malaria occurs yearly in the two villages a quarter of a mile up the hill, which obtain their water entirely from this "Ain" or spring: the turning up of the soil was of course blamed for the outbreak.

I shall shew elsewhere that, in all probability, the larvae and pupae of *Anopheles* have been taken into the houses with drinking water in many cases. They may thus become disseminated and give rise to malaria at points situated a mile or two from their breeding-places. I have not as yet seen this possible mode of dissemination of *Anopheles* referred to.

Tyre. The town itself is practically free from fever, though there are several places a few miles away where infection only too easily occurs. At the mouth of the river Leontes or Kasmiyeh, early in June, I found several boys with fever and enlarged spleens, and on my return on August 3rd the number was still larger, though the population here is very small. Time did not allow of adequate search being made here, but *Anopheles* larvae were found further up the river the day before.

It was at Ras el Ain, four miles south of Tyre, however, that I found the largest percentage of infection. Here amongst 19 children of 12 years and under, I found the spleen markedly enlarged in every case, though two of the children were only 5 and 7 months old respectively. The average enlargement of the spleen in 9 children of 6 years and under was 10 cm. below the ribs; of 10 children over 6 and under 12 years, somewhat less, viz. 9.2 cm., and two of the former were, as I have said, only 5 and 7 months old at the time of our visit in August.

This bears out the observations made in Africa by Christophers and Stevens¹ as to the relative frequency of infection in children, though they enjoy no immunity from fever, from which I have known them die.

¹ Reports to Malaria Committee of the Royal Society, 3rd series, 1900.

Any case of doubtful enlargement was excluded as not evidently malarial. I used the enlargement of the spleen as the most reliable test of the relative unhealthiness of any given place. It was rarely found possible to obtain blood-films from children, not ill at the time, owing to the objections of their parents.

A. maculipennis (3 specimens) was found in a native reed hut at Ras el Ain, from which two malarial patients came, and their breeding-place was probably not far distant in marshy pools, formed amongst the sand, as the water of the Ras el Ain runs to the sea.

Several young men from this place were evidently suffering from malarial anaemia, and a few of the older people who came round had splenic enlargement or were as the Arabs say "*muthooleen*" or "*spleened*." A few of the boys in the crowd were found to have no evidence of malaria, but on enquiry these in every case came from Tyre or villages on the hills.

Plain of Acca. Near the Ras en Nakûra, a native house furnished *Anopheles maculipennis* abundantly (20 or 30) with a few *A. superpictus*, and in the green slimy ditch outside the corresponding larvae were found at once by Dr Gould of Acca, who had come to meet me. On a subsequent occasion the other houses were all found to contain *Anopheles*. The guard of soldiers placed here is changed every month, for as they explained everyone gets fever after 10 days. Time did not allow of the examination of many villages of this district known to be malarious, but in the houses situated in gardens on the banks of the river Belus imagines of *A. maculipennis* were found 10 to 30 in a room, hanging as is frequently the case from cobwebs, with which the ceilings are freely adorned. The only cases of enlarged spleen which, during four or five years' work in Acca, came from the neighbourhood of the town, were from this spot or from similar gardens called El Bahjeh.

Near here there is much uncultivated ground and six of the houses are deserted because the "air is bad." The man in charge of the flour-mill protects himself by lighting a nightly bonfire before retiring to rest; the blackened beams and his bleared eyes bear eloquent testimony to the fact, but otherwise he seemed healthy enough.

At Tell Kurdaneh, near the source of the river Belus, *A. maculipennis* was most abundant in the living-room of the mill, probably an old Crusading fort—the miller, a former patient of mine there, is apparently outgrowing his fever, his spleen being however still very large.

Haifa. This place like the town of Acca is mostly free from malaria, nor could I detect *Anopheles*, but time did not allow of an

examination of the banks of the Kishon to the east, from near which most of the cases come.

Athlit. By the kindness of Dr Coles of the English Hospital at Haifa, I was enabled to drive down the coast. At Athlit, from which place many malarial patients come to Haifa, after a long search I found one *Anopheles* but no other *Culicidae*. The only person who applied to me for medicine was a woman whose spleen was enormously enlarged. The marshes near this wretched village, built amongst the ruins of the Crusading Castellum Peregrinorum, are now almost dried up.

Zammarin. Though on an elevated site malaria is endemic here, and in the water trickling from the well on the road-side below the colony two *Anopheles* larvae were found. The water supply of this Jewish colony (founded by Baron Rothschild) is excellent, being pumped up by steam from deep wells.

Khūdeirah. On June 29th driving on from Zammarin along the Jaffa road, in two hours we came to springs of water flowing slowly through marshy ground past Khūdeirah towards the sea—the colony lying on a sandy hill to the south-west. Two million *Eucalyptus* trees have been planted here. In puddles formed by feet of buffaloes in the black mud larvae of *A. maculipennis* were found abundantly, amongst *Spirogyra* and *Lemna*. Further on I found more larvae, and on reaching the colony *A. maculipennis* was found hanging from the roof of the very shed used for potting the young *Eucalyptus* trees raised from seed. They were also found in a stable close by.

Even before reaching the deep (50—70 ft.) well in the centre of the colony we could see that the houses are built far apart (50—100 yds.), the ground being planted with rows of *Eucalyptus*, twelve deep, now of large size, *i.e.* fully grown.

The soil here is very dry, red and sandy, and the nearest marshes are from $\frac{1}{4}$ to $\frac{1}{2}$ a mile distant. The colony is half deserted, malaria is rife, and blackwater fever occurs here yearly, and is said to have caused the death of 150 Jews in the last 20 years.

In my opinion, it is plain that the *Eucalyptus* trees have not done the slightest good, and perhaps only harm. Had half the money spent on planting these been used in carrying out hydraulic works the place could not fail to be much healthier. The natives suffer much less from malaria than the Jews, and hardly at all from blackwater fever. I could not hear of any cases of this disease when we were there, but in

some years 15 to 20 cases have occurred in a very limited population, chiefly Jews emigrated from Europe.

The colony of Tantura on the coast is now deserted on account of the severe malaria and blackwater fever, of the occurrence of which I obtained good proof from Dr Weiss, now of the colony at Zammārīn, and from Dr Kaufmann, formerly of Haifa, who also treated cases of the disease.

From Acca I proceeded to Safed, and thence to Isbeid, another Jewish colony, which was described as the most unhealthy place in the district. Isbeid (or Issôd) lies practically at sea-level and has a mixed population of Jews (in the colony), Fellaheen natives and emigrants from the village of El Khiam, Bedaween (several tribes), encamped on the plain on either side of the colony, and Moorish Arabs from Tunis, living in huts of reeds or papyrus matting. The Jewish colony has been founded about 20 years, and during the whole of this time malarial fevers have proved a great scourge: blackwater fever also occurring commonly in some seasons. In one year there were 19 cases in six weeks: its incidence is chiefly from October to January. Last year there were only four cases. Most of these facts I obtained from Dr Weiss. At the present time the dispenser affords medical advice, and in his opinion 90 % of the population have enlarged spleens. I was unable to take a census of the population.

Here the breeding-ground of *Anopheles* larvae was, in summer at any rate, entirely in the water oozing out of the pebbly beach of the lake, above its present level. The lowness of this is partly caused by the light rains of last winter, but more especially by the lowering of the bed of the Jordan at the end of the lake by the Sultan, to whom most of the land near here belongs. Further measures for deepening and widening the bed of the river are shortly to be undertaken, which will in time bring large tracts of land under cultivation, but at the same time certainly result in fresh breeding-places for *Anopheles* larvae. Near the native village of Et Teleil is a piece of typically marshy ground covered with water in the winter, but the oozing margin of the lake is the source of *Anopheles*, for the colony itself is quite dry all summer.

Our tent was pitched for 12 days under the *Eucalyptus* trees, *E. rostrata* and *resinifera*, the climate being unsuitable for *E. globulus*. Mosquitoes, almost entirely *A. maculipennis* and *A. superpictus* with very few *Culex*, were common, and could nearly always be found in our tent, the dark navy blue lining of which formed a shelter, as did the

dry herbage under the *Eucalyptus*, where they were always to be found.

Until this year quinine has been given free to the colonists: now it is sold at cost price, under the new management by the Trustees of Baron Hirsch.

If ever quinine has had a good trial amongst a far from savage people, it is here, and I was informed by Dr Weiss of the incredible doses of quinine formerly given to patients ill of blackwater fever: 20 grammes in a single day in one case, in another 12 grammes a day for a week, and with subsequent recovery. Pernicious attacks are frequent, and the parasite most commonly found by me was of the aestivo-autumnal type.

At various places round the lake-side and along the course of the river, such as the Jisr Benât Yakoob *A. maculipennis* and *superpictus* were abundant, *A. pharoensis* being present but in small numbers.

Banias. Of 36 children examined at this place 86 % had enlarged spleens, a higher percentage than at Isbeid, and here as there malarial cachexia with splenic enlargement is by no means infrequent in grown-up people. *A. superpictus* larvae were found in semi-stagnant pools in the rocky river bed, and imagines of both species were found in the houses and in our tent. As at Isbeid the malignant form of the parasite was common, and crescents were found in two cases, but not in large numbers. The Bedaween tents in the valley contained as many mosquitoes as the native houses: 15 *A. maculipennis* being caught in one tent. The tribes who live permanently in the valley of course suffer much less than those only recently arrived.

From Banias we made a short journey to Shibáh, which lies over 5000 ft. above the sea. Here, as also last year, I found *A. maculipennis*, though the nights are far from warm. For the most part the population of this village is very healthy, but we saw two cases of cachexia, probably of malarial origin.

Rasheyat-el-Wady. This place, about 4000 ft. above sea level, is usually free from malaria, but from time to time it has been visited by extremely severe epidemics of ague. The last outbreak occurred 9 or 10 years ago, and in it eleven people are said to have died in one day. Making full allowance for exaggeration, there is no doubt that a severe epidemic of malaria occurred, the nature of the disease being well known to every native.

After long search one of the springs of water below the town was found to contain *Anopheles* larvae and *Spirogyra*. The other collections

of water, mostly of rain-water, only contained *Culex*. The spring in question, named the 'Ain en Nijmi, is used for drinking when the larger public well has run dry, which happens every autumn, and one can easily understand how larvae and pupae may be carried in jars of drinking water to every house in the town. In dry seasons the water has often to be brought from much greater distances on the heads of women. It seems to me that not much credence need be placed in the assertion that a marsh in the valley nearly two miles away which is flooded every winter is the cause of fever. I found *Anopheles* larvae also in two springs on the side of the road leading to Hasbeyah, where too in the pools of the Hasbâny river *Anopheles* larvae occurred in plenty. In the town we had not time for a thorough examination of likely places. An English lady in charge of the British Syrian schools was suffering from malaria at the time of our visit.

From Banias we also made an excursion into the papyrus marshes of the Huleh¹ Valley, three or four hours distant. Our camping place for the night was probably two miles distant from any human habitation, and here we found that the sole species of *Anopheles* was *A. annularis*, subsp. *pseudopictus* Grassi. Other species of *Anopheles* were conspicuous by their absence. A species of *Culex*, *Toeniorrhynchus richardii*, was also found, but of 230 Culicidae caught, over 80% proved to be *Anopheles*.

Owing to the trouble given by an unruly horse all three of us were bitten severely during the night by hundreds of *A. pseudopictus*, and on two other occasions one or both of us were bitten freely in similar places in or near the marsh: none of us have since suffered from ague. I might add here that we never observed *Anopheles* to bite during the daytime, when *Culex fuscatus* bit freely.

In conclusion I would cite the following observations made by others:—

Dr Gould found *Anopheles* larvae in a cistern in a garden at Nablus in June 1901. Dr Masterman writes to me from Jerusalem that tertian and quartan fevers are common there, and that in August these form $\frac{3}{4}$ of the patients treated by him, excluding ophthalmia. At Silwân (Siloam) malaria is rife, and almost all the children of the Yemen Jews there have enlarged spleens. Dr Masterman has not as yet searched for *Anopheles*.

¹ "El Hûleh" in Arabic means the terrible or frightful, probably with reference to its unhealthy character.

Conclusions.

The observations I have made in Palestine would appear to warrant the following conclusions :—

1. Malaria is rife in every place where *Anopheles* represents the majority of the mosquitoes present in native dwellings.
2. Malaria occurs mainly but not entirely amongst children, and these shew definite signs of infection in enlarged spleens and fever.
3. Malaria occurs sporadically or not at all in places where an unsuccessful search was made for *Anopheles*.
4. Blackwater fever only occurs in the most malarial districts and chiefly amongst Europeans, *i.e.* immigrant Jews.
5. Æstivo-autumnal fevers, shewn by the unpigmented ring forms, greatly predominate, though quartan, a few tertian, and mixed infection also occur.

STUDIES IN RELATION TO MALARIA.

II.

THE STRUCTURE AND BIOLOGY OF ANOPHELES

(*Anopheles maculipennis*).

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(*Continued from page 484, Vol. I.*)

Resting Position of the Imago.—Geographical Distribution of the Species.—Habitat.—Modes of Dissemination and Migratory Flights.—Hibernation of the Imago.—Longevity.—Oviposition, and the Influence of Food thereon.—Parthenogenesis.—Numerical Proportion of Sexes.—Number of Generations during a Season.—Food.—Influence of Heat, Cold, Light, Colour, and Sound.—Sense of Smell and Taste.

ALL recent authors (Ross, Grassi, etc.)¹ are agreed that an essential generic difference exists between *Anopheles* and *Culex* with regard to the position assumed by the imago when at rest. Whereas in *Anopheles* the proboscis and body are almost in a line, so that the insect has not inaptly been compared to a brad-awl (Christy), *Culex* presents a hump-

¹ See Bibliography, p. 75, and p. 484, vol. I., this *Journal*; as also at the end of this paper.

backed appearance, as figured by Waterhouse (Howard, 1900, p. 34), and, apart from this, the axes of the proboscis and body do not correspond, but form an obtuse angle with each other, the proboscis pointing more towards the surface upon which the insect rests. The structure of *Anopheles* is altogether more slender and graceful, and the legs are considerably longer and more slender than those of *Culex*. In both genera the imago rests either upon four or six legs, the more frequent position being the former, the hind pair being held so that the tarsal joints are elevated and, especially in *Culex*, curved upward. In *Anopheles* the tarsi may be slightly turned upward, but usually they are straightened, the bend being at the femoro-tibial articulation. In a box in which the insects are confined we have frequently opportunities of observing a peculiar rotatory motion of the hind legs, whose function would appear to be largely tactile. When approached by a flying insect the hind legs are used to push away the intruder, and when in search of food the hind leg appears not infrequently to guide the insect. This was especially seen when the insects were fed upon banana or sugar and milk, for in walking about, if the tarsal extremity of the hind leg touched upon a moist surface, the insect immediately turned round and proceeded to feed. Judging from their extended position whilst flying the hind legs doubtless act as balancers. As far as we are aware, Ross was the first to draw attention to the habit of "standing on its head" which characterises *Anopheles*, although we now know that different species behave somewhat differently in this respect, some pointing away at a greater angle from the surface than do others. Sambon and Low (1900) have criticised the statement of Ross and Austen (1899) that the position of the genus *Anopheles* is characteristic, i.e. "practically at right angles to the surface," on which it is resting, for they found *A. maculipennis* resting at but a slight angle, *A. superpictus* rested at most at an angle of 45° when the hind feet were raised, whilst *A. pseudopictus* held its body almost at right angles. Gray (Dec., 1900, p. 1820) of St Lucia observed *A. albitarsus* to rest at an angle of 45° , and Christy (1900, p. 1821) has found three out of four species of *Anopheles* in Bombay to assume the position described by Ross. The statement by Sambon and Low, that the "resting position of mosquitoes loses all importance" is therefore misleading. The position is distinctly an aid in recognizing the members of the genus, even if they rest at but slight angles to the surface. In any case Waterhouse was right in the terse statement, "Whatever may be the attitude of *Anopheles*, it is all in one line. *Culex* is angular, hump-

backed." In *Culex*, moreover, the abdomen may be parallel to the surface, or its extremity may be directed toward the surface, and owing to its shorter legs its body hugs the surface more closely than that of *Anopheles*.

In the case of *A. maculipennis*, Howard (1900, p. 31) finds that when it rests on a vertical wall its body stands at an angle of 10° to 20° , at times at 30° to 40° , sometimes almost parallel, in the latter case a hind or middle leg being not infrequently broken off. In our observations made on males and females we have noticed the fly resting on vertical surfaces at angles corresponding to those given by Howard. When hanging from a horizontal surface, the angle measured 50° to 70° . When resting on a horizontal surface, the body was nearly parallel, slightly elevated posteriorly (10° or less) or even slightly depressed.

Geographical Distribution of A. maculipennis.

In the paper by Nuttall, Cobbett and Strangeways-Pigg (*Journ. of Hygiene*, vol. I., p. 5) it is recorded that *A. maculipennis* has been found in England, Wales, Scotland, Ireland, Scandinavia, Germany, Austria, Russia, Holland, Denmark, Italy and the adjacent islands, Canada, and the United States. Howard (June, 1901, p. 113) states that this species is found almost everywhere in the United States, for he has seen specimens from the States of New Hampshire, Connecticut, New York, the District of Columbia, Maryland, Virginia, Florida, Texas, Louisiana, Indiana, Illinois, Minnesota, and Oregon, whereas Packard recorded its presence at Brunswick, Maine, in 1861-63. Cropper (p. 49, this *Journal*) has found the species in Palestine.

Habitat.

There is reason to believe that *Anopheles* do not as a rule wander far from their breeding-places, and consequently we shall find the imagines near to those places which we have described as suitable habitats for the larvae (vol. I., p. 70). Some writers have claimed that the distribution of certain species of *Anopheles* coincided with that of human habitations, but this has certainly not been supported by further experience. (See further under Hibernation.)

Modes of Dissemination.

In addition to the data mentioned on p. 8, vol. I. of this *Journal*, with regard to the mode of dissemination of mosquitoes, through winds,

trains, ships, and streams we would record the following facts. Howard (1901, p. 20) says he is incredulous regarding the supposed long flights of mosquitoes since he has had occasion in many cases to observe that mosquitoes breed close to localities where their breeding-places had not been suspected. Weeks, of Bayside, Long Island, does not agree with Fernald's observation (cited by us, vol. I.) for he finds many breeding-places in the immediate vicinity of Cold Spring Harbour. Fermi and Lumbao (22 Aug., 1900, p. 180) referring to *Culicidae* in Sassari, state that the females do not travel far because they obtain sufficient food from sucking the blood of man and animals. Howard (1901, p. 17) writes: "In the summer of 1900, Mr W. J. Matheson, living at Lloyd's Neck, Long Island (U.S.A.), a spot formerly infested by mosquitoes to an extraordinary degree, by intelligent exterminative measures succeeded in practically stopping the breeding of mosquitoes upon this narrow neck of land. It resulted that his house was mosquito-free until toward the end of August. Then, after a gentle and continuous wind of two or three days' duration, specimens of another kind of mosquito put in their appearance in large numbers. The explanation was obvious. These mosquitoes had traversed a strip of water forming one of the entrances from Long Island Sound to Oyster Bay, for a distance of a mile or a little more, aided by the gentle and continuous wind."

In the Public Health Reports published by the U.S. Marine Hospital Service (vol. xvi., 9 Aug., 1901, p. 1792) two communications relative to the dissemination of mosquitoes are published by members of the Service. P. C. Kalloch (surgeon) writes from the Gulf Quarantine Station "that the captain of the ship *America*, arriving in quarantine July 24, stated that mosquitoes came aboard the vessel on the previous night, at a distance of at least 10 miles from land, the nearest point being Chandeleur Island. The opinion prevails in this locality that mosquitoes are blown by south-west winds from the Louisiana marshes to this island, a distance of 10 to 20 miles, and the experience of this summer seems to accord with the opinion. Mosquitoes were very few in number until the middle of July, when, after several days of south-west winds, the number was vastly increased. At that time there was no other local condition to explain the sudden increase."

The second communication is one by H. C. Cumming (passed assistant surgeon), regarding the presence of mosquitoes on board the Spanish bark *Maria Blanquer*, which arrived at the South Atlantic Quarantine Station from Rio Janeiro. The master was positive that

there were no mosquitoes on board until the twenty-second day out, when some were noticed in the water tank when opened. The water had come from Rio Janeiro. The mosquitoes became so numerous on the journey as to constitute a veritable plague, the people on board having to cover themselves to have rest. The U.S. Quarantine officer reports, "When the forecandle was opened, after fumigation, mosquitoes could be scooped up by the hand."

Further instances of the supposed influence of railways in the dissemination of mosquitoes are given by Howard (1901, pp. 25—28), who quotes letters from various correspondents. One of these claims that there were no mosquitoes in the city of Mexico before the railways were built from Tampico and Vera Cruz, mosquitoes being very numerous in both of these low-lying places. Lounsbury, Government Entomologist at Cape Town, wrote that the railway had been responsible for the dissemination of mosquitoes in many parts of South Africa, and another correspondent "gives details of a precisely similar introduction of mosquitoes into a high lying Missouri town." Howard cites an observation at Winchester, Virginia. This place was at one time a favourite summer resort, mosquitoes being almost unknown until the establishment of a night train service during summer at a time when the water-works of the town were extended without provision for adequate drainage. "As a result, with the arrival of the mosquitoes from Baltimore there was a plentiful supply of standing water all through the city, and conditions were thus perfect for the development of mosquitoes in enormous numbers." It does not seem to us that this last case is convincing, for given a suitable supply of water, the local mosquitoes, however small in numbers, would have sufficed for the stocking of the numerous new breeding-places.

Migratory Flights.

Hitherto no migratory flights have been observed in connection with *Anopheles*. In view of the very positive statements of some authors to the effect that the flight of the *Culicidae* is very limited, it seems desirable to cite the following observations recorded by Howard, in which however neither the genus nor species of *Culicidae* involved is mentioned. The conditions which bring about such migrations are unknown. Howard (June 1901, p. 22) cites a personal letter from J. D. Mitchell, of Victoria, Texas, in which that gentleman states that he has twice had occasion to observe mosquito migrations. On the first occasion (October 1879), "A fairly strong easterly wind had been blowing

for three days; on the evening of the third day the mosquitoes arrived, flying high about fifty feet, and looking like a cloud or mist coming from Carancahua Bay. At the ranch they set everything on fire that had blood in it, and all work was suspended by unanimous consent... little or nothing was done for nearly five days; by this time the main body had passed, though plenty remained to make everything uncomfortable for about two weeks. This migration was from east to west and the line was about three miles wide..." It would appear as if the mosquitoes had originated from a marsh eighteen square miles in extent, situated at a distance of 35 miles as the crow flies. This flight crossed two expanses of water which were respectively five miles and one mile broad.

The second migration occurred from the same place in the year 1886. This time the flight was narrower but denser. "They clouded the sky, bent down the grass with their weight, and made all drift-wood and ground the same colour. All stock left the shore and went north outside the line of marsh. The wind was light and from the south, and did not affect the mosquitoes in their flight, which was westward; the main flight was low, ten or twelve feet high and always in the same direction." The flight lasted almost three days, stragglers being left behind. Enquiries showed that the flight extended over a distance of 50 or 60 miles. This flight crossed four expanses of water, which measured respectively 1, 2, 3, and 4 miles.

Similar migrations have been observed in connection with other insects, scattered observations being found in entomological literature. The phenomenon may possibly be due to the over-stocking of a given locality by a species. It would seem probable that the sound produced by the flight of numerous insects of one species might exert an influence upon the formation of these swarms, for as we shall see later sound plays a very important part in the life of mosquitoes.

Hibernation of Imago.

We have referred already to the seasonal occurrence of the imago and larvae and to the hibernation of the larvae of *Anopheles*, and have seen that apparently only the larvae of *A. bifurcatus* survived the winter in cold climates. Entomologists agree that *Culicidae* hibernate as imagines. Nuttall wrote (1899, p. 111) that Finsch (1876) made observations on the Siberian Tundras which led him to believe that the imago of *Culex* hibernated beneath the moss. "Sterling (1891) saw

mosquitoes at Mackinaw, Sault Ste. Marie in March, 1844, when the snow, which lay 2 to 4 feet on the ground, was being melted by the sun. The insects appeared in thousands, and bit his party until sundown. Stewart (1891), of North Carolina, saw mosquitoes appear in swarms in March, when several feet of snow lay on the ground. They "literally blackened the banks of snow in sheltered places. These were evidently the insects of the previous summer which were wintering over. The Indians told us that the mosquitoes lived over the winter, and the old ones are the most annoying to them." Westwood (1872-76) noted the hibernation of gnats in his house at Oxford, the insects being troublesome during winter evenings. Wade (1884) saw *Culex ciliatus* Fabr. hibernate in his cellar; Aaron (1890) and Young (1881) have made similar observations.

Grassi (1900, pp. 84-86) says that he has never found the males of *A. maculipennis* hibernating, but only the females, all of these being fecundated. The development of the eggs was retarded by the cold, and advanced with the onset of warm weather, when the female would again begin to bite. The imago was very frequently encountered in houses, stables, chicken-houses, especially in heated apartments. In central and southern Italy they were found to hibernate in cabins and grottos, although less numerous in these situations. The imagines begin to disappear in February, and vanish to a greater extent in March, when at times none are to be found. These insects have presumably flown out, and died after depositing their eggs. He states (p. 47) that but few *A. bifurcatus* hibernate as imagines. A few *A. superpictus* were found hibernating in grottos.

Mr Theobald informed us last year that the imagines of *A. maculipennis* which have hibernated in England disappear early in May, "no doubt to oviposit and then die." We have found this species hibernating in the cellar of a house in Cambridge, and have caught them occasionally during the winter, as has also Mr Verrall at Newmarket (*v. tables on p. 16, vol. 1. of this Journal*). Annett and Dutton have since (27 April, 1901) reported the finding of hibernating imagines in similar situations in north and mid-Cheshire during the month of February. Howard (1900, p. 12) states that Dr Thayer of Baltimore observed *A. crucians* and *A. quadrimaculatus* (*A. maculipennis*) hibernating in enormous numbers in barns near New Orleans, clustering on the roofs and on the walls. We find, like other observers, that the female imagines live longer in captivity as winter approaches, and it is presumed that such insects would successfully hibernate under natural conditions.

Longevity of the Imago.

From what has just been stated there appears to be no evidence that the male imagines hibernate, and this sex has in all probability a much shorter life than the female. Grassi (1900, p. 58) states that he could only keep *Anopheles* alive for a month in the laboratory. Howard (1900, p. 11) could only keep *A. maculipennis* alive for eight days in confinement during the summer, but Woldert kept them alive for 15 days on banana alone. In the autumn they lived for 50—60 days in confinement, these being insects which would probably have hibernated. We succeeded in the summer of 1900 (July—August) in keeping females alive on a diet of banana and water for 14 to 56 days, it being found essential to keep the atmosphere moist and the food fresh, both of which conditions are apt to be neglected. We have also noted that the females were able to live longer in captivity as winter approached. We have found that unfed males and females, confined immediately after their escape from the pupa-case and maintained at about 20° C., survived two to three days. That *Culicidae* may survive longer unfed is indicated by the observation of Veazie (Howard, 1901, p. 10) of New Orleans, who found some to survive unfed for five days, whereas Mitchell in Texas claims that he has seen them survive unfed for 21 days. In the last case the conditions of moisture are not stated, and no information is given regarding the species, the temperature, nor if the insect started to fast on a full stomach.

There is every reason to believe that the imagines may survive much longer in their natural state than they do in confinement. We shall presently refer to the influence of the character of the food upon longevity.

Oviposition.

With the exception of Kerschbaumer (1901, p. 54) nobody has claimed to have observed the process of oviposition and as far as we know no one has witnessed copulation. After numerous attempts made between 8 p.m. and 2 a.m., Kerschbaumer found that both *Anopheles* and *Culex*, at any rate in captivity, only oviposited in the early morning hours. He witnessed the process but once in *Anopheles*, thrice in the case of a species of *Culex*. He does not however describe the process (excepting in so far as he says the insect rested directly upon the water) and what he says of *Culex* has already long been known. He found that unfed

captive *Anopheles* hardly ever seemed to lay a full complement of eggs. A female laid a batch of 146 eggs, and subsequently laid six more. We have already referred (vol. I., pp. 49—51) to the behaviour of the eggs after being laid. Observations upon captive insects have shown that the nature of the food supplied has a considerable influence upon fertilization and oviposition. Thus Ross, Annett and Austen (1900, p. 21) found that if *Anopheles* males and females were placed together and fed on fruit no fertilization nor oviposition took place even after weeks of confinement. The contrary was the case when the females were fed on blood. Fertilized females laid a second batch of eggs after receiving a meal of blood, but did not do so when kept on a fruit diet. Grassi (1900, p. 85) had seen the female of *A. maculipennis* suck blood some hours after oviposition and the insect survived for some days in the laboratory. He thought that the process of oviposition might be repeated several times. Both Grassi and Ross and his colleagues have concluded that blood is essential for the reproduction of the species which they studied. Austen (30 March, 1901) writes, "The experience of the members of the Sierra Leone Expedition of the Liverpool School of Tropical Medicine showed that eggs are laid only by female *Anopheles* which have had a natural feed of blood, and that naturally fed specimens invariably laid eggs after two to three days." Specimens reared from the egg, and kept for some time with males, then isolated in test-tubes and allowed to suck blood, never laid eggs. The inference drawn is that a meal of blood is a necessary preliminary for fertilization. According to the Report of the Expedition, "The following law is likely to hold good for the *Culicidae* which feed on man, at least for the common species:—Although these gnats can live indefinitely on fruit, the female requires a meal of blood both for fertilization and the development of her ova. In other words, the insects need blood for the propagation of their species." It was found that "previously fed and fertilized insects would lay a second batch of eggs after a second meal of blood without a second fertilization, but never laid a second batch of eggs without a second meal of blood," that is, one fertilization sufficed for several batches of eggs, but one meal of blood for only one batch of eggs.

Annett, Dutton and Elliott (1901, p. 37) fed some *Anopheles* on blood, others on banana, varying the conditions of the experiments. They reached conclusions confirmatory of the preceding, viz. that a purely vegetable diet is insufficient for the propagation of the genus *Anopheles*; that blood is necessary for the development of the ova; that blood must be available regularly at least every two days for the

ova to develop; that the power of propagation is acquired in a very short time from the appearance of the imago, and is vigorous during the whole life of the insect when the latter is fed on blood; that one fertilization by the male suffices for a considerable period of ova production. They found that unfertilized fully-developed ova may be carried for as long as four weeks by the female.

That other conditions may influence oviposition has been shown by Bancroft (5 June, 1901), who observed mosquitoes in captivity which frequently did not lay eggs on clean water, whereas they often oviposited in putrid water. Bancroft does not state to what genus these mosquitoes belonged.

Although there can be no doubt as to the necessity of blood for the propagation of the species under the conditions of the experiments quoted above, it seems to us quite premature to lay down any law which would apply to the life-history of these insects under natural conditions. In the above experiments the mosquitoes as a rule appear to have only had the choice between the banana and blood. The fact that the insects did not propagate on banana and did on blood does not prove that blood is a condition *sine quâ non*. Before we can reach such a conclusion we must know more about the food which these insects may seek in nature, and on this point we have very little information. That the insects, at any rate in limited numbers, frequently have access to blood is of course clear from the mere fact that they are necessary for the distribution of malarial and certain filarial parasites. But this is no scientific proof of blood being necessary to the propagation of the insects. We certainly need exact and further studies upon the natural food of the *Culicidae*.

Parthenogenesis.

The only direct reference to parthenogenesis which we have found is that of Howard (1901, p. 4), who states that Kellogg in California observed it in a species of mosquito, the genus of which is not mentioned. A female insect which escaped from the pupal covering whilst in captivity almost immediately laid eggs, which hatched out, the larvae almost reaching maturity. "There was no other mosquito in the jar and certainly no mating." Annett, Dutton and Elliott (1901, p. 241), in their Report on the Malaria Expedition to Nigeria, state that some captive *Anopheles* (species not mentioned) were seen to lay eggs without having been fertilized, but in this case the eggs did not hatch out.

Numerical Proportion of Males and Females.

Rees (March 1901, p. 290) states that "When mosquitoes are bred in captivity the males, as a rule, hatch out first, and in greater numbers than the females." We have found no similar statement elsewhere, and the observations we have made do not tend to confirm his observation. The proportion of males to females has always appeared to us to be fairly equal, and we have counted the sexes on several occasions.

Number of Generations during the Season.

Observations made by Italian observers indicate that between three and four generations of *Anopheles* may be developed during a season. Kerschbaumer (1901, p. 85) observed four generations to occur in Austria. The first appeared in April and the beginning of May, the second appeared in the beginning of June and first week of July, the third appeared in the end of July and the first three weeks of August, the fourth between the middle of September and the middle of October. He could find no larvae of *A. maculipennis* after the middle of October. In these cases the number of generations was determined by the finding of young larvae. Basing his calculation upon the assumption that a female may lay 150 eggs, and assuming further that all the descendants lived, he figured that the number of descendants calculated up to the fourth generation would number 31 millions. Our observations tend also to show that about four generations may develop during a season in the neighbourhood of Cambridge.

Food of the Imago.

In view of the enormous numbers of *Culicidae* which occur in certain countries, especially where there are few animals from which they can suck blood, naturalists have hitherto agreed in considering them essentially vegetable feeders. In any case but an infinitesimal number ever have an opportunity of sucking blood, and in the vast majority of cases it would appear that it is but the female which feeds on blood. Dimmock (1881) considered that the male could not suck blood, he wrote, "Upon anatomical grounds I believe that male mosquitoes take liquid food, although I never dissected their stomachs to see what this food was. They have mouth-parts and pharynx developed sufficiently to suck liquids..."

The statement has been made that a few species of *Culicidae* exist in which the males suck blood. Stiles informed Howard (1901, p. 37) that he was once bitten near Leipzig by what was apparently a male *Culex nemoralis*. We shall certainly gain more knowledge on the subject now that so much attention is being given to this interesting group throughout the world. In the case of *Culex salinus* Ficalbi, it is stated that the male has mouth-parts like the female and that it is capable of sucking blood. It is quite possible in the case of the observation made by Stiles that the insect was an anomaly. Certainly in the case of *Anopheles maculipennis* the female only has mouth-parts adapted for penetrating the skin (see pp. 464—467, vol. I, this *Journal*).

In Nuttall's monograph (1899, p. 113) notes will be found regarding the food of *Culicidae*. Various species have been seen to feed on bananas, melons, and other fruits. Smith, of Rutgers College, New Jersey (Howard, p. 34), has seen mosquitoes (*Culex sollicitans*?) feeding on wild-cherry blossoms: "So abundant were they that he captured hundreds by sweeping his net over the blossoms." Joly (May 1901, p. 258) says that he has seen mosquitoes rise in clouds from over-ripe mangoes, lying on the ground, as also from bananas and oranges, although they preferred the first. Nuttall states that Murray (1885) saw *Culex* destroy very young trout, the adult insect, as Aaron puts it, "literally sucking out their unsuspecting little brains before they could escape." Combes (1896) made a similar observation on the Island of Anticosti. The mosquitoes attacked the "petits poissons filiformes" and sucked out their heads. When released the little fish turned belly upward and floated on the water, dead. These mosquitoes were also seen to attack an allied species of insect while the latter were issuing from the pupa-case. At this time the fly is still very soft, and it is readily sucked out by the other species which attack it. Howard (1901, p. 34) was informed by Veazie that he had seen *Culicidae* feeding on the soft-skinned pupa of *Cicada*, whilst Hagen in the north-western States saw one feed on the chrysalis of a butterfly. Brakeley informed Howard that he had seen mosquitoes sucking the blood of a black terrapin.

Referring to *Anopheles*, we find that Grassi (1900, p. 84) states that the males never contained food (cap. III.) and that he had never seen male *A. maculipennis* feed. His observations however are not final, for we have repeatedly seen them feed ravenously on bananas, sugared cherries, dried figs, sugar, and milk. Howard (1900, p. 12) states that the males of other *Culicidae* have been also observed to feed, sipping up water,

molasses and beer, and Gray of St Lucia (1900) noted that *Culex pipiens* (?) had a predilection for port wine. We see then that it is abundantly established that the male *Culicidae* do feed upon nutrient and even intoxicating fluids.

A number of authors have dwelt upon the importance of *blood* as a normal article of diet for the female *Anopheles*. Speaking of *A. maculipennis* Grassi (1900, pp. 82—83), says that the ordinary food of the female is blood, although Ficalbi has seen them suck up vegetable juices, and even the contents of latrines, and Grassi has seen them feed on unripe maize, water, and sugar and water. He kept them on this diet for a month at 15° to 25° C., but observed that their numbers decreased. He concludes, "In breve si può dire che alle femmina degli Anofeli la dieta vegetale non basta e il sangue è indispensabile." He says that they only appeared to suck the blood of warm-blooded animals, usually that of mammals, exceptionally that of birds, also that they are more attracted to large than to small animals. The same author (1900, p. 1307) mentions elsewhere in a footnote that he has kept *A. maculipennis* alive for a long time (how long, not stated) on a diet of melon. James (1900, p. 535) in India found that he could only keep *A. rossii* and another undetermined species of *Anopheles* alive for 4 or 5 days on a banana diet, whereas if he occasionally fed them with blood they lived for 14 to 18 days. Ross, Annett and Austen (1900, pp. 20—21) saw both the males and females of *A. funestus* and *A. costalis* feed on banana. The female digested a meal of blood in one to three days depending upon the size of the meal. Grassi (1900, p. 84) observed that the female *A. maculipennis* required 10 or more days to dispose of a meal, when the thermometer registered 15° C., but only 40—50 hours in summer-time. Bancroft (5 June, 1901) has found dried dates to be an excellent food for mosquitoes, better than bananas, which he was the first to suggest: "they do not get rotten or even mouldy; and there is no necessity, as with banana, to change for fresh every three or four days; a single date hung in the mosquito cage will serve throughout the experiment however long it may last. Mosquitoes fed on dates live longer, and many species that will not live in confinement more than three days on banana, *e.g.* *Anopheles nussimus*, Skuse, *Culex vittiger*, Skuse, thrive on dates and live for upwards of a month." Our observations are in substantial agreement with these.

Rees (March, 1901, p. 292) observed that the *Anopheles* with which he worked bit more readily at times when the hand which was offered them had been previously immersed in warm water. We have had no

difficulty in persuading *A. maculipennis* to bite in confinement. We have fed them on human blood and by placing a rabbit within the tent in which the insects were confined. They did not feed upon a toad (*Bufo vulgaris*) nor upon a large earthworm although the flies were hungry. A male was seen to feed upon the body juices of a crushed spider. Rose-buds covered with *Aphides* offered a great attraction, doubtless because of the sugary substances present, which were moistened with dew. Both sexes feed ravenously on sugar and milk or sugar and water, the females becoming very fully distended. (See further above under "Oviposition.")

Kept in a suitable vessel of which the atmosphere is sufficiently moist, or in which water or fresh banana is kept and renewed, the female remains alive for a considerable time. Females were kept alive in captivity during the summer (July and August) for two to eight weeks. During this time their abdomens were frequently seen to be distended with banana juice. A number of flies were repeatedly fed with blood. When the temperature ranged between 20° and 26° C. they willingly sucked blood every two or three days. After a full meal the abdomen is greatly swollen and tense. At first the blood gives the abdomen a bright red colour, which changes to a lake as digestion proceeds. (See vol. I., Plate X.). After a full meal or even whilst sucking, a drop of intestinal contents is frequently expelled from the anus.

Effect of Heat and Cold upon Anopheles maculipennis.

We have referred elsewhere to the effect of cold in retarding the development of the larvae (vol. I., p. 69, this *Journal*) and we have seen that they became more active during warm weather, feeding more continuously and growing more quickly. During cold weather it has commonly been observed that the imagines of all the *Culicidae* become torpid, and cease to be troublesome. Insects caught in cold cellars during the winter become active when warmed, and it has been frequently noted that they will then readily suck blood. In other words the *Culicidae* behave very much like other insects with regard to their activity under varying conditions of temperature. The larval forms as we have shown (pp. 451—453) of *A. bifurcatus*, at any rate, may resist degrees of cold considerably beneath zero Centigrade. As far as we know no recorded observations have as yet been made in this respect upon imagines.

Behaviour of the Imago to Light.

The preference of some species of *Anopheles* for dark and shady places has been repeatedly noted by observers in various parts of the world. During the day-time the imagines congregate in caverns, grottos, beneath the shade of trees and bushes, within the precincts of dwellings and barns, beneath bridges, etc. It is true that they have been observed to be present during the daytime, but frequently they are absent except on clouded days. They fly about chiefly towards sunset and until sunrise. We are able to confirm the earlier statements of Grassi in this respect, and similarly Sambon (26 Jan. 1901) writing of *A. maculipennis* which he studied in the Roman Campagna in July and August writes that the imagines appeared "very punctually a few minutes after sunset and disappear again a few minutes before sunrise." We have repeatedly had occasion to watch the insects (*A. maculipennis* and *A. bifurcatus*) we had in captivity observing this rule. When boxes were placed near the window the insects retreated into the shadiest parts and remained quietly resting throughout the day. About the time of sunset a loud buzzing came from the boxes, and the insects promptly fed upon any substances that were present and which they generally neglected throughout the day. At night when confined in a room, tent, or gauze net, they invariably crowded to the side which was illuminated by a lamp, apparently seeking an exit in this way. If confined within a lamp-chimney they fly towards the end which is held towards the light, evidently with the same object. It has been claimed as a matter of common experience in mosquito-ridden countries, that a room can be rid of the insects to a certain extent by keeping it dark and placing a light in an adjoining apartment, the door being left ajar.

However Ross, Annett and Austen (1900, p. 38) state that lights in a room tend to prevent gnats from biting "not by attracting them, as many suppose: but more probably by alarming them."

Colour.

The behaviour of the insects towards various colours has not as yet received sufficient attention. Whilst engaged with experiments upon the influence of shade and colour we came upon a few data cited in the recent literature.

Austen (March 1901, p. 341) writes, "If the walls of the room be white-washed, with a dark dado, it is interesting to note that the

insects will always be found upon the dark strips, and never on the white portions of the wall." Buchanan (April 1901) in India notes that "The men who collect the living *Anopheles* say that the *Anopheles* hide in a black coat, but avoid a white coat, so they hang up one or two black coats in the Hospital Ward" when they desire to catch the imago. Neither Austen nor Buchanan say anything about the influence of colour. The first as far as we know to refer directly to the influence of colour is Joly (May 1901, p. 259) who made observations on mosquitoes in Madagascar. He states, without saying what genus, that mosquitoes there were more attracted to black than to red soil, or to white sand. Persons wearing black shoes and socks were more bitten than when these articles of apparel were white. Brown clothes protected less than those of white or blue. He states that the natives of Madagascar know the attraction black offers to mosquitoes and for this reason hang up a black cloth on the rafters of the room for the insects to collect upon. Joly observed that a yellow haired dog was very much less bitten than a black one. For the same reason the natives are more bitten than the whites, although they suffer less from the after effects.

It seemed to us to be a matter of considerable practical utility to determine what influence, if any, colour exerted upon a known malaria-bearing species of mosquito. And we deem our results sufficiently striking to make it worth the while for those who are engaged in similar studies abroad to take the matter up systematically. Our experiments certainly indicate that *Anopheles maculipennis* is attracted by some colours and repelled by others, a matter which would have its *practical application in the choice of the colour of clothing and the interior of rooms* in malarious districts. We are moreover inclined to believe that suitably constructed coloured boxes, or *colour-traps*, might be of practical utility in and about houses infested with mosquitoes. By periodically closing the boxes and sweeping out the contained insects into a receptacle, or, possibly by rendering the interior of the boxes sticky a considerable number of mosquitoes might be destroyed.

Our experiments were made in a large gauze tent which had been erected within a disused photographic establishment, the one end of the tent ending against large windows into which the sunlight poured on bright days. Large stone basins were placed on the floor for the *Anopheles* to breed in, the stock being renewed from time to time.

It was noticed at the beginning that when one entered the tent in dark grey clothes, that the imagoes frequently flew up and settled on the dark cloth, but that they never did this when the person entering

the tent was clothed in white flannels. To test the influence of colour, a number of pasteboard boxes were taken which measured 20 by 16 cm. and had a depth of 10 cm. The boxes were lined with cloth, having a slightly roughened surface, to which the insects could comfortably cling. All of the fabrics had a dull—not shiny—surface, and each box was lined with a cloth of different colour. The boxes were placed in rows upon the floor and upon each other in tiers, the order being changed each day after the observations had been made. The interior of the boxes was moderately illuminated by light reflected from the surface of the white tent. On 17 days during a month beginning with the middle of June, we counted the number of flies which had accumulated in the boxes. Counts were actually made on 17 sunny and cloudy days, and with the following result :

Colour of Box							Number of <i>A. maculipennis</i> counted in each box during 17 days.
Navy blue	108
Dark red	90
Brown (Reddish)	81
Scarlet	59
Black	49
Slate grey	31
Dark green (olive)	24
Violet	18
Leaf green	17
Blue	14
Pearl grey	9
Pale green	4
Light blue (forget-me-not)	3
Ochre	2
White	2
Orange	1
Yellow	0
							512

We see from the above table that dark blue was most attractive, the other colours being less and less attractive in the order of numbers given. A marked fall in the number of insects resting in the boxes begins with the "pearl grey" box. Pale green, light blue, ochre, orange, and yellow, especially the last two colours seemed to repel the insects. The karki-coloured uniform at present in vogue should offer advantages besides invisibility to human foes! These observations on colour were described by one of us in a short note which appeared in the *British Medical Journal* (14 Sept. 1901).

Mr J. Cropper of Mount Ballan, Chepstow, who read the above note wrote to us (17 Sept., 1901): "Seeing your article on Colour Selection by *Anopheles* reminds me that I found the dark navy-blue lining of my tent this summer (in Palestine) extremely attractive to mosquitoes, almost entirely *Anopheles*—and when the sun got hot I always noticed an increase in their numbers, presumably as they came from the herbage and trees near by. No one ever slept in the tent, and I never found *Anopheles* bite in the daytime¹."

Moreover Dr H. E. Durham has since informed us that whilst he was studying yellow fever at Pará, Brazil, he was much less bitten about the feet than was his late companion Dr Myers. Dr Durham wore ochre coloured socks, Dr Myers black ones².

Hearing.

It would appear to be generally accepted that the organs of hearing are situated in the antennae of the *Culicidae*.

In 1855 Johnston, of Baltimore, U.S., wrote: "That these parts themselves are, in some instances, concerned in collecting and transmitting sonorous vibrations, we hold as established by the observations we have made particularly upon *Culex mosquito*; while we believe, as Newport (*Trans. Entomol. Soc. II.*) has asserted in general terms, that they also serve as tactile organs." Referring to the bulbous enlargement at the base of the antennae, and noting their larger size in the male insect, he says, "The space between the inner and outer walls of the capsule, which we term confidently the auditory capsule, is filled with a fluid of moderate consistency, opalescent, and containing minute spherical corpuscles, and which probably bears the same relation to the nerve as does the lymph in the *scala* of the *cochlea* of higher animals. The *nerve itself*, of the antenna, proceeds from the first or cerebral ganglion, advances towards the pedicle of the capsule in company with the large *trachea* which sends its ramifications throughout the entire apparatus, and, penetrating the pedicle, its filaments divide into two portions. The central threads continue forwards into the antenna and are lost there; the peripheral ones on the contrary radiate outwards in every direction, enter the capsular space, and are lodged for more than

¹ See also p. 54, this *Journal*.

² Note whilst going to press: According to a newspaper report which we have seen, our experiments have promptly led to a practical application in the United States Army in the abandonment of the regulation shirt of navy blue in favour of white shirts for service in malarial districts where mosquitoes abound.

half their length in *sulci* wrought in the inner wall or cup of the capsule."... "The intra-capsular fluid is impressed by the shock, the expanded nerve appreciates the effect of the sound, and the animal may judge of the *intensity*, or *distance*, of the source of sound, by the *quantity* of the impression: of the *pitch*, or *quality*, by the consonance of particular whorls of stiff hairs, according to their lengths; and of the *direction* in which the modulations travel, by the manner in which they strike upon the *antennae*, or may be made to meet either *antenna*, in consequence of an opposite movement of that part."

"That the male should be endowed with superior acuteness of the sense of hearing appears from the fact, that he must seek the female for sexual union either in the dim twilight, or in the dark night, when nothing save her sharp humming noise can serve him as a guide." He also notes that the male mosquito is more difficult to catch. The coloured plate which accompanies the interesting contribution gives a very fair representation of the head of a male *Culex* and of the structures under consideration.

An important paper in this connection is that of Mayer (1874) who was familiar with the publication of Johnston. He cemented a male *Culex* with shellac to a glass slide and placed it beneath a $\frac{1}{5}$ objective. He then "sounded successively near the stage of the microscope a series of tuning-forks with the openings of their resonant boxes turned towards the fibrils," and saw that a Ut_4 fork, of 512 vibrations per sec. set certain fibrils in vigorous vibration, whilst others remained comparatively at rest. He measured the amplitudes of the vibrations of the fibrils under the influence of the sound emitted by various tuning-forks. We shall only cite four out of nine such measurements: the Ut_2 fork caused a vibration of .0042, the Ut_4 fork of .0504, the Mi_4 fork of .0126, the Ut_5 fork of .0168 mm. When the forks were vibrated with lower intensity a corresponding lessening in the amplitude of the vibration was noticeable. Different hairs were seen to vibrate to different notes. He also observed that when the sound came from a direction corresponding to the line continued through the long axis of the antennary hairs that vibration ceased. This led him to suppose that the antennae could register the *direction* whence the sound comes. When he observed the antennae under the microscope he found that vibration ceased, when the hairs pointed towards the source of sound, and on drawing a line in the direction in which the hair pointed, he found that "it always cut within 5° of the position of the source of sound."

"The antennae of the male mosquito have a range of motion in a

horizontal direction, so that the angle included between them can vary considerably inside and outside of 40° , and I conceive that this is the manner in which these insects during night direct their flight toward the female. The song of the female vibrates the fibrillae of one of the antennae more forcibly than those of the other. The insect spreads the angle between his antennae, and thus, as I have observed, brings the fibrillae, situated within the angle formed by the antennae, in a direction approximately parallel to the axis of the body. The mosquito now turns his body in the direction of that antenna whose fibrils are most affected, and thus gives greater intensity to the vibrations of the fibrils of the other antenna. When he has thus brought the vibrations of the antennae to equality of intensity, he has placed his body in the direction of the radiation of the sound, and he directs his flight accordingly; and from my experiments it would appear that he can thus guide himself to within 5° of the direction of the female."

An attempt which we have made to study the effects of electromagnetically excited tuning-forks upon male *A. maculipennis* gave unsatisfactory results, this being possibly due to their having been confined for some days in small boxes together with females. We hope however to pursue the subject next season.

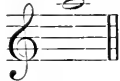
Sound produced in Flight.

According to Howard (1901, p. 14) the sound during flight is "apparently produced, as with flies and other dipterous insects, not by the rapid vibration of the wings, but by the vibrations of a chitinous process in the large tracheae just behind the thoracic spiracles. These vibrations are produced by the air during respiration." He furthermore states that the sound produced in its flight is higher in *Culex* than it is in *Anopheles*, adding that "the villain in the play has usually a bass voice."

Our experiments do not support Howard's assertion, with regard to the wing not producing a note, for we have found by cutting off more and more of the wing, that the sound decreased in volume, the note rising progressively. When the wing was cut off quite closely, a very high-pitched note of slight intensity remained, this as we supposed being produced by an internal apparatus such as Howard indicates. It may however be due to respiratory movements which are exaggerated through the efforts at flight, the sound is not produced by the insect in repose. We found that the males gave a higher-pitched note than the females, and that the note was higher in both sexes when

they had fed; the greater the meal, the higher the note. Of four unfed females three gave notes within a quarter of a tone of 264 (*i.e.* of 240 to 270 vibrations), the fourth female gave an abnormally low note of about 175 vibrations. Four other females were arranged in the order of the distension of the abdomen by food, the last being largely distended, these gave notes corresponding roughly to 264—281—297—317 vibrations or according to the musical scale, the notes:



Three unfed males gave exactly the same note, *viz.* corresponding to 880 vibrations  immediately after feeding one gave the note

A \sharp , another which had fed well B \sharp . The unfed males were more closely concordant than the unfed females, the latter varying over about a semitone. Mr J. W. Capstick, M.A., Fellow of Trinity College, Cambridge, to whom we are greatly indebted for making these ear determinations for us by means of tuning-forks, was not certain that the note given by the males was not one of 440 vibrations. Overtones were obviously strong and it sounded at times as if there were a faint note of 440 vibrations overshadowed by a strong one of 880.

The obvious explanation of the higher note given off by the males is that their wings are markedly narrower and shorter than those of the females. Although a female *Culex pipiens* gave a higher pitched note than a female *A. maculipennis*, we are not at all sure that it was not simply due to the smaller size of the former insect. The male of this species of *Culex* certainly gave a higher pitched note than the female.

But few recent writers refer to the sense of hearing in *Culicidae*. Grassi (1900) states that persons are more liable to be bitten by *Anopheles* when engaged in conversation than when silent. Joly (20 May, 1901) in Madagascar observed that mosquitoes were decidedly affected by music. He states that if he played a stringed instrument all the previously quiescent mosquitoes in the room began to fly about, and if the window were open they flew in from the outside. The same observation was made in the open, in the evenings, whether music was played in the dark or near a lamp. The mosquitoes (genus not mentioned) gathered about the player in great numbers.

Howard (1901, p. 15) was informed by Mr A. De P. Weaver, an

electrical engineer of Jackson, Miss., that "while engaged in some experiments in harmonic telegraphy, in which a musical note of a certain pitch was produced by electrical means, he was amazed to find that when the note was raised to a certain number of vibrations per second, all mosquitoes, not only in the room where the apparatus was, but also from other parts and from the outside, would congregate near the apparatus and would be precipitated from the air with astonishing force, striking their bodies against the apparatus. He states that he therefore covered a large surface with sticky fly-paper and after sounding the note for a few seconds captured all the mosquitoes in the vicinity. He then devised an apparatus to electrocute them. A section of wire window-screen with the paint removed was mounted on a board and small pins were driven between the meshes, the heads coming flush with the surface of the screen. All the pins were connected together electrically, the whole forming one electrode of the secondary coil of an induction coil, while the wire screen formed the other electrode. An alternating current of high potential was then passed and when the note was sounded the insects precipitated themselves against the screen and were immediately electrocuted. Mr Weaver, unfortunately, does not state whether the males were captured in this way."

In a brief note in the *British Medical Journal* (12 Oct. 1901, p. 1101) Ross states that he has been informed by Mr Brennan, of Jamaica, that he has seen mosquitoes there "respond to such sounds as a continuous whoop or hum," and he goes on to say "I have tried the experience lately, and find swarms gather round my head when I make a continuous whoop."

Our attention has moreover been drawn to a letter by Sir Hiram S. Maxim in *The Times* of October 29, 1901. We herewith quote the essential parts of the letter:

"In 1878 I made and erected an apparatus for lighting the grounds of the Grand Union Hotel at Saratoga Springs, New York, by electricity. The lamps employed were rather large and each was provided with its own dynamo machine. One of the lamps worked something like a telephone and gave out a note the pitch of which corresponded exactly with the strips on the commutator passing under the brushes of the dynamo machine. Some of the other lamps would occasionally give off a musical note, but only for a few minutes at a time. With this one, however, the note was practically constant, and no adjustment of the carbons had the least effect upon it. One evening whilst examining this lamp I found that everything in the immediate vicinity was covered with small insects. They did not appear to be attempting to get into the globe, but rather into the box that was

giving off the musical note. Upon a close examination of these insects I found that they were all the same kind—viz., mosquitoes, and, what is more, all male mosquitoes. Although there were certainly 200 times as many female mosquitoes on the grounds as males, I was unable to find a single female mosquito that was attracted in the least by the sound. When the lamps were started in the beginning of the evening every male mosquito would at once turn in the direction of the lamp, and as it were face the music, and then fly off in the direction from which the sound proceeded. It then occurred to me that the two little feathers on the head of the male mosquito acted as ears, that they vibrated in unison with the music of the lamp, and as the pitch of the note was almost identical with the buzzing of the female mosquito the male took the music to be the buzzing of the female. I am neither a naturalist nor an entomologist, still I was much interested in this peculiar and interesting phenomenon. I wrote down a full account of it at the time and sent it to a scientific paper, but it appeared to be too stupid to find a place in that particular publication. However, it now appears that others have stumbled across the same thing. A very interesting experiment may be easily made in the following manner:—Obtain a tuning-fork which gives a musical note as much like the hum of the female mosquito as possible. If you strike this fork within 20 ft. of a male mosquito he will at once turn about, face the music, and erect the two little feathers on his head, something after the manner of a cockatoo."

We have collected here a number of perfectly independent observations made with respect to sound upon various species of *Culicidae* in different parts of the world. It is quite evident that the matter requires careful study, for it is not impossible that the knowledge gained might be ultimately put to practical use.

Smell and Taste.

That insects are often particularly sensitive to odours is a matter of wide experience, and this holds also for such as suck blood. As stated by Nuttall (1899, p. 86) the common flea (*Pulex irritans*) is repelled by the smell of the horse, and this insect as also the bed-bug are attracted or repelled by the body-odour of certain individuals. That mosquitoes are more attracted to certain individuals than to others has been frequently noted, and we know that a variety of odorous substances afford protection against the attacks of these insects. So far practical experience has taught us a good deal about repellent odours, but little about those which *attract*, and it would seem to us distinctly useful if such could be discovered, for combined with sticky substances they might be very useful in ridding rooms of these pests. Of the repellent odours we might mention a few, such as the oils of pennyroyal, eucalyptus, peppermint, tar; whilst lemon-juice, kerosene, tincture of pyrethrum, sulphur etc. have also been used to afford protection. Joly

(1901, p. 259) has recently observed in Madagascar that mosquitoes (genus not stated) were markedly attracted to dried fish.

We have no knowledge as to the situation of the organs of smell. It is not impossible that odours may be appreciated within the tracheal system.

We know nothing regarding the sense of taste, but that it exists can scarcely be doubted; it is striking how fond the insects are of sugary solutions.

POSTSCRIPT.

NOTE to p. 452, (vol. I., this *Journal*) Mr Theobald informs us that the *Anopheles* larvae found at Wye in December (reported upon by Annett and Dutton) were those of *A. bifurcatus*. In his opinion this species always lives through the winter in the larval form; he has never observed hibernating imagines. We have already cited various observations with regard to this species on the page referred to above.

Correction to be made on p. 479 (vol. I., this *Journal*). Cross out the sentence on lines 22—23, reading "The absence of scales.....generic importance," and read instead "Mr Theobald informs us that the abdomen is usually nude of scales, but they may be present in the form of narrow spindle-shaped ones, in some species of *Anopheles*. He finds that the abdomen may be densely scaled for example in *A. pharoensis* Theo., *A. kochi* Dönitz, etc."

(*To be continued.*)

LITERATURE.

The following list contains a number of recent papers not cited in the text, these are marked by an asterisk, the contents being indicated by their titles or by a brief note. The object in citing them is to render the bibliography of the subject as complete as possible.

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*SMITH, T. (15 Jan., 1901), Note on the occurrence of *Anopheles punctipennis* and *A. quadrimaculatus* in the Boston suburbs. *Journ. of the Boston Soc. of the Med. Sciences*, vol. v., pp. 321—324.

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(Communication of but a few lines, stating that they have found these two species in India where they had not been hitherto described. They prevailed in the planting districts of the Duars. The "interest of the discovery lies in the fact that they are the two species of *Anopheles* which carry malarial infection in Tropical Africa." They are common in India where blackwater fever is most frequent.)

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*WASIELEWSKI, VON (1901), Ueber die Verbreitung und künstliche Uebertragung der Vogel malaria. *Archiv f. Hygiene*, vol. XLI. Heft 1, pp. 68—84. (Experiments upon the transmission of proteosomal infection by artificial inoculation to various birds. Reviews the literature; gives numerous interesting facts; he found, contrary to Koch and Ruge, that infected canaries did not acquire immunity after recovery, but suffered from chronic malaria lasting up to 11 months, very few parasites being present in the blood, their presence being only demonstrated by inoculating the blood into fresh animals.)

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ON THE PROTECTIVE SUBSTANCES OF IMMUNE SERA.

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THE theories of Ehrlich⁽¹⁾ on the Substances and Mechanisms concerned in the production of Immunity have opened up a very wide field for speculation and research. His view on the formation of the Antitoxins forms the most satisfactory and useful hypothesis which has been produced upon this subject, and is constantly receiving fresh support from the accumulating evidence of numerous observers. But of the more recent developments of his theories as applied to antimicrobial agencies some of the details appear to be at present insufficiently established. With certain of these details it is here proposed to deal.

Methods.

In all the experiments to be recorded the cultures used were 48 hour cultures of a given variety of the *B. typhosus* on an agar surface of approximately constant area. For injection they were washed off the agar surface with a known volume of ordinary culture bouillon, and emulsified by thorough shaking. The dose to be administered was then measured volumetrically, accuracy of measurement being sought by increasing the dilution of the emulsion where small doses were required. The injections were made intraperitoneally with a hypodermic needle. Serum when given was mixed and injected with the infecting dose of the bacillus. The weights of the guinea-pigs used were determined before injection to within 5 grammes, and all the injections given were calculated for the actual body-weight. They are stated in the accounts of the experiments in terms of the amount per 100 grms.

of weight. Care was exercised to obtain animals as similar as possible in age and weight. An autopsy was made on every animal which died. The Immune-serum used throughout was the antityphoid serum of Tavel obtained from horses.

The Theory of the Specialism of the Addiment.

It has been pointed out by Ehrlich, Wassermann, and others that if the amount of Immune-serum necessary to protect an animal of given weight against a single M.L.D. (minimum lethal dose) of a given bacterium be determined, and a similar animal be now injected with three times this amount of serum and three times the M.L.D. it will die, not being sufficiently protected by the quantity of Immune-serum given. Moreover Roux and Vaillard⁽²⁾ had shown for tetanus what has been fully confirmed for other infections also (*e.g.* for cholera by Pfeiffer), that an infected animal treated with Immune-serum may rapidly die of the disease in question although it has been given such an amount of serum as to render its body-fluids capable of protecting other animals against infection by the same bacterium. That is to say, in general, that if an animal receives more than a certain maximum dose of an infecting agent it will die, whatever the quantity of the Immune-serum that is employed in the endeavour to protect it. And seeing that in this second type experiment the Immune-body is obviously present in sufficient quantity—since the fluids of the dead animal can protect another animal—Ehrlich attributed the phenomena observed in these experiments to a deficiency of the addiment. And on this deficiency of addiment he founds the theory which it is our purpose to consider.

Taking first the position of Pfeiffer, that both the substances necessary to protection are present in Immune-serum in an inactive form, and become active after injection into the body of an animal, it is evident that if the addiment injected is rendered available and active by the body-cells of the animal concerned it should be possible to supply this addiment in sufficient quantity by the injection of sufficient Immune-serum. But this is by the experiment quoted found to be impossible. Ehrlich therefore came to the conclusion that the foreign addiment is useless to the animal injected; in other words, that an animal can only make use of its own addiment, which is limited in amount, or of that of other animals of its own species: in fact that addiment is special to the species.

It remained possible, however, that exceptions might exist and that

certain species of animals might possess addiments acceptable to certain other species. And this appeared to be the case. These exceptions, therefore, become of great importance, for only by a careful search could there be found for each particular species the addiment acceptable not only to the Immune-body for a given bacterium but also to the animal itself. And more especially for serum-therapy in man Ehrlich regards this search as of prime value and of pressing importance for the immediate future.

The writer⁽³⁾ has already elsewhere expressed the opinion that this theory of the extreme specialism of the addiment is both unnecessary and misleading; and that the facts are capable of better explanation on the view that owing to its extremely labile nature the addiment of antibacterial action becomes broken down and degenerated, and rapidly disappears from artificially separated serum. That is to say, that addiment is *absent* from the ordinary antimicrobial sera. That the position of Ehrlich is not by any means satisfactory is shown by the following considerations among others. In every observation of the so-called Pfeiffer phenomenon made by means of the Immune-serum of other animals in the peritoneal cavity of the guinea-pig, the school of Pfeiffer believes that the inactive addiment of this serum is rendered active by the agency of body-cells, that is to say, a foreign addiment is satisfactory to the reaction in the guinea-pig. Again Wassermann⁽⁴⁾ found that in infection of guinea-pigs a satisfactory addiment for this animal, and for the Immune-serum of dogs which was used as the protective agent, existed in the fresh serum of the ox—an animal of a species as widely different from the guinea-pig as from the dog; while Ehrlich and Morgenroth discovered similar relations in their work upon the haemolysins. Taking all the facts into consideration I came to the opinion formulated in a previous paper that (stored) antibacterial sera possess in general no addiment, and that in the Pfeiffer phenomenon as in the other experiments in question the animal concerned has to make use of *its own* addiment, which is limited in quantity, and therefore may prove insufficient for the work required of it. And evidence in support of this contention can be adduced from various directions, as I now proceed to show.

In the former of the two type-experiments quoted, in which the fact that three serum-equivalents¹ do not protect against 3 M.L.D. is attributed to lack of addiment; the position held by Wassermann appears to have

¹ By the term serum-equivalent is meant the amount of serum necessary to protect 100 grammes of guinea-pig against a single M.L.D.

originated in a mathematical error. The result is in reality due to a deficiency in the amount of Immune-body injected.

For if d represent the M.L.D. then if the M.L.D. has been determined within 10 %, say, of the total dose $\frac{9}{10}d$ was the nearest dose less than d which proved not fatal. That is to say, an unimmunised animal has natural immunity to destroy a dose of $\frac{9}{10}d$.

Hence in the case of the first injection of one M.L.D. and one serum-equivalent, the latter was only engaged in actively protecting against—that is in providing Immune-body against $\frac{1}{10}d$. And since the least amount of serum necessary for protection against the M.L.D. constitutes the serum-equivalent of that M.L.D. we may say that one serum-equivalent is the protective equivalent of $\frac{1}{10}d$. Now in the second case $3d$. and 3 serum-equivalents are injected. And the 3 serum-equivalents are the protective equivalents of $3 \times \frac{1}{10}d$, that is of $\frac{3}{10}d$ only. Also the animal itself can if the remainder of the dose be neutralised by serum-protection (but only then) deal with an amount of the infective material equal to $\frac{9}{10}d$. There remains however a dose of $(3 - \frac{3}{10} - \frac{9}{10})d$, i.e. $1\frac{8}{10}d$, against which no protective arrangement whatever is made and obviously the animal must die.

If the M.L.D. had been determined to within nearer limits than the 10 % supposed, the result is all the more favourable to the argument, if within wider limits only, somewhat less so. But in any case the greater the number of M.L.D.'s and the number of serum-equivalents given the larger will be the dose of the bacterium against which no protection whatever is afforded, and the more rapidly and certainly will the animal die. And if in this experiment the serum-equivalent had been determined not for the M.L.D. but for some multiple of this and the experiment continued as before, then by a similar train of reasoning the serum given would fail to be protective when the doses of infective material and serum were progressively multiplied as before. While therefore in the experiment with large infecting doses and unlimited amounts of Immune-serum there is exhibited a deficiency of addiment, in this experiment there is no evidence of deficient addiment but only of deficiency of Immune-body. In proof of this assertion may be quoted the following series of observations.

The M.L.D. of a particular variety of typhoid bacillus, one of the varieties actually employed in the preparation of the serum itself from horses, was found to lie between the limits 0.05 and 0.075 of a 48 hour culture on an agar surface of approximately constant area for each 100 grms. of guinea-pig. The serum-equivalent of this M.L.D. (taken always as 0.075

of a culture) was 0.025 c.c. of serum per 100 grms. of guinea-pig, and 0.02 c.c. per 100 grms. was not protective. The animals themselves could therefore deal with a dose of 0.05 of a culture per 100 grms. Hence the serum-equivalent of the M.L.D. was engaged in protection against 0.025 of the culture only: it was therefore the protective equivalent or true serum-equivalent of this amount of culture. I shall speak of this volume of serum as the serum-unit. A simple calculation now showed that if the view maintained be justified 2 M.L.D. would require serum protection against $[(2 \times 0.075) - 0.05]$, *i.e.* 0.1 culture and this would be afforded by $\frac{0.1}{0.025}$, *i.e.* 4 serum-units,

3 M.L.D. would similarly require $\frac{(3 \times 0.075) - 0.05}{0.025}$ serum-units, *i.e.* 7 serum-units,

4 M.L.D. would similarly require $\frac{(4 \times 0.075) - 0.05}{0.025}$, *i.e.* 10 serum-units, and generally n M.L.D. require $\{1 + 3(n - 1)\}$ serum-units.

And in general if d be the M.L.D. and e the largest dose not fatal, then the number of serum-units required to protect 100 grms. of guinea-pig against n M.L.D. of the bacterium will be given by the formula $\frac{nd - e}{d - e}$,

that is $\left[1 + (n - 1) \frac{d}{d - e}\right]$ serum-units.

The following were the experimental results obtained with a series of guinea-pigs.

{ Guinea-pig 1 received 1 M. L. D. and 1 serum-unit, the animal							
{	"	2	"	1	"	"	recovered
	"		"	"	"	"	died in 16-18 hrs.
{	"	3	"	2	"	"	recovered
	"	4	"	2	"	"	died in 18 hrs.
{	"	5	"	3	"	"	recovered
	"	6	"	3	"	"	died in 14-16 hrs.
{	"	7	"	4	"	"	recovered
	"	8	"	4	"	"	died in 18-20 hrs.
¹ {	"	9	"	5	"	"	died within 14 hrs.
	"	10	"	5	"	"	" " "
¹ {	"	11	"	6	"	"	" " "
	"	12	"	6	"	"	" " "

¹ Deficiency of addiment.

And these results have been confirmed¹. Hence up to and with four M.L.D. and the corresponding number of serum-units theoretically required on our hypothesis the animals are completely protected, but not by any less amounts of serum. I submit therefore that the view which has been here put forward is sufficiently established; and have in all the subsequent experiments calculated the amount of serum which contains a quantity of the Immune-body sufficient for protection by this formula. This quantity I have called the *theoretical serum requirement* of the animal for the given dose of the infective agent.

It was apparent in the above series of experiments that a deficiency of addiment first began to occur on the exhibition of 5 M.L.D. together with the appropriate number of serum-units. It seemed quite certain therefore that by working with doses of ten times the M.L.D. inaccurate results from accidental coincidence could be avoided in an investigation of the addiment. I therefore proceeded to examine the theory of its specialism as follows.

Experiments were made,

- (a) with the *fresh* blood-serum of certain unimmunised animals;
- (b) with the same sera when they had been kept for some days after separation in an ice-chest, at a temperature approaching zero;
- (c) with the fluids obtained by digesting such old sera with *fresh* blood-clot which was broken up and added to them, the whole being kept for one or two hours in the incubative chamber, and the fluid afterwards centrifugalised from the fragmented clot as described more fully later.

The results obtained are illustrated in the subjoined series of experiments.

EXPERIMENT (a).	Result
Guinea-pig 1 received 10 M.L.D. and 28 serum-units ² of Immune-serum 	The animal died within 16 hrs.
Guinea-pig 2 received 10 M.L.D. and 28 serum-units of Immune-serum and 1 c.c. <i>fresh</i> ³ Rabbit serum per 100 grms. 	died within 16 hrs.
Guinea-pig 3 received 10 M.L.D. and 28 serum-units of Immune-serum and 2 c.c. <i>fresh</i> Rabbit serum per 100 grms. 	recovered

¹ In another series of experiments with a more virulent variety of the *B. typhosus* the deficiency of addiment appeared at the fourth M.L.D.

² The theoretical serum requirement according to the formula obtained above $\frac{nd - c}{d - c}$.

³ Fresh serum here means serum used within 10 hours of the bleeding of the animal from which it was obtained.

EXPERIMENT (a).

Result

{	Guinea-pig 4 received 10 M.L.D. and 28 serum-units of Immune-serum and 1 c.c. <i>fresh</i> Ox serum per 100 grms.	died in 20-24 hrs.
	Guinea-pig 5 received 10 M.L.D. and 28 serum-units of Immune-serum and 2 c.c. <i>fresh</i> Ox serum per 100 grms.	recovered
{	Guinea-pig 6 received 10 M.L.D. and 28 serum-units of Immune-serum and 1 c.c. <i>fresh</i> Pig serum per 100 grms. 	died in 26-28 hrs.
	Guinea-pig 7 received 10 M.L.D. and 28 serum-units of Immune-serum and 2 c.c. <i>fresh</i> Pig serum per 100 grms. 	recovered

Hence the freshly won serum of the three different animals examined—the rabbit, ox, and pig—can all supply the missing addiment for guinea-pigs, and this addiment is ‘satisfactory’ to the Immune-body of the immune-serum of the horse. We have here five distinct species of animals concerned, and it seems reasonable to conclude that Ehrlich’s theory of the extreme specialism of the addiment to its own species of animal is untenable.

I have suggested on the other hand that owing to its extreme *lability* this addiment is absent from *stored* serum, and this I found to be the case. Thus I examined serum of the rabbit, ox and pig after it had been kept for some days in the ice-chest. In most cases it was a week to ten days old, but in the case of the ox serum only two to three days old, when thus made use of in the experiments from which the following are taken.

EXPERIMENT (b).

Guinea-pig 1 received 10 M.L.D. and 28 serum-units ¹ of Immune-serum		all dead within 18 hrs.
{	Guinea-pig 2 received 10 M.L.D. and 28 serum-units of Immune-serum and 2 c.c. <i>old</i> Rabbit serum per 100 grms.	
	Guinea-pig 3 received 10 M.L.D. and 28 serum-units of Immune-serum and 3 c.c. <i>old</i> Rabbit serum per 100 grms.	
{	Guinea-pig 4 received 10 M.L.D. and 28 serum-units of Immune-serum and 2 c.c. <i>old</i> Ox serum per 100 grms.	
	Guinea-pig 5 received 10 M.L.D. and 28 serum-units of Immune-serum and 3 c.c. <i>old</i> Ox serum per 100 grms.	
{	Guinea-pig 6 received 10 M.L.D. and 28 serum-units of Immune-serum and 2 c.c. <i>old</i> Pig serum per 100 grms.	
	Guinea-pig 7 received 10 M.L.D. and 28 serum-units of Immune-serum and 3 c.c. <i>old</i> Pig serum per 100 grms.	

¹ The theoretical serum requirement of 10 M.L.D.

The sera therefore which had been kept for a few days had lost the power they previously possessed of supplying the deficient addiment. That is, they had lost their addiment. Hence anti-bacterial addiment rapidly disappears from a stored serum.

Further, the addiment of the fresh sera was found to be destroyed by heating for half-an-hour to a temperature of from 52°—53° C. (nos. 1 and 2 below), though heating the Immune-serum for an hour to this temperature or to 56° C. produced no apparent alteration in its protective power (nos. 3 and 4 below). Thus

Guinea-pig 1 received 10 M.L.D. and 28 serum-units of Immune-serum and 2 c.c. per 100 grms. <i>fresh</i> Ox serum	recovered
Guinea-pig 2 received 10 M.L.D. and 28 serum-units of Immune-serum and 2 c.c. per 100 grms. <i>fresh</i> Ox serum heated for 1 hour at 53° C.	died within 20 hrs.
Guinea-pig 3 received 10 M.L.D. and 28 serum-units of Immune-serum heated 1 hour at 56° C. and 2 c.c. per 100 grms. <i>fresh</i> Ox serum	recovered
Guinea-pig 4 received 4 M.L.D. and 10 serum-units of Immune-serum heated 1 hour at 56° C.	recovered

Thus, while the addiment of fresh serum was destroyed by heating to this temperature, the action of the Immune-serum was unaltered. Hence I conclude that the latter contains no addiment to be destroyed. The addiment which is concerned in anti-bacterial action on the *B. typhosus* is therefore *not* particularly 'special to the species,' but is of an extreme lability, and hence is *absent* from any but fresh serum whether the latter be obtained from immunised or unimmunised animals.

The Nature and Origin of Addiment.

In one and the same serum the addiment is not the same for all the anti-cellular reactions possible to that serum, but different. Thus as regards the haemolytic action of goats' serum Ehrlich has shown the existence of two kinds of addiment at least, the one destroyed rapidly at a temperature of 56° C., the other only slowly and still resistant at a temperature equal to 65° C. And we have seen that the addiment of anti-bacterial action here examined is still more labile.

Now Ehrlich has put forward the following view of the position and function of addiment on the normal body. It is, he claims, a ferment produced and discharged into the blood by certain cells—which are not

further specified—to serve the purposes of general cell-metabolism. It is taken up from the plasma by means of an addimentophil group of the side-chains of the body-cells, and thereby enables them to carry on their normal cycle of nutrition by its power of splitting up the large molecules of nutritive material extracted from the blood and rendering their assimilation possible.

But anti-microbial serum has *in vitro* no bacteriolytic action. It contains Immune-body, but offers no evidence of the possession of any such ferment as Ehrlich presupposes, for as we have seen the bacteriolytic addiment is absent from stored sera. Such serum may be rendered active by cellular action, as for example by a sojourn in the peritoneal cavity (Pfeiffer), by the addition of fresh normal serum, which contains leucocytes and their products—Bordet's⁽⁵⁾ phenomenon,—or by the addition of other leucocytic fluids (Hahn⁽⁶⁾). The activity thus obtained is removed by heating at 56° C., a process which as has been shown destroys the addiment; it may be again restored by the addition of fluid containing leucocytic products (Bordet, Hahn.) Moreover a definite relationship exists between the mass of the leucocytes added and the degree of bactericidal addimentary power obtained (Bordet). Again, a bacteriolytic pleural exudate has been made entirely inactive by the removal of its leucocytes,—active again on their replacement (Denys and Havet⁽⁷⁾). Additional evidence in the same direction was obtained in a continuation of the experiments already quoted, in the observations now to be recorded.

In these experiments I took normal sera of the rabbit and the pig, which had been found to have lost their addiment by keeping, and used them for extracting *fresh* blood-clot of their own, or other species of animals. The procedure was as follows: fresh clot was broken up and rapidly centrifugalised, and the resulting fluid decanted. The amount of serum then remaining in the clot was insufficient to affect the accuracy of the subsequent experiment, in view of the comparatively large amount of fluid next employed. This consisted of a volume of the old serum calculated to be greater than the volume of serum normally corresponding to the quantity of clot which had been centrifugalised. This volume of old serum was now added to the clot and thoroughly mixed with it in a mortar. The mixture was then placed in the incubator for an hour or two, and the fluid subsequently separated by centrifugalisation and tested for protective power, that is for the possession of addimentary action. It was then found that the fresh clot had yielded addiment to the previously addiment-free old serum of

animals, even of a different species from that of the animal which supplied the clot. I quote the following experiments.

EXPERIMENT (c).		Result
Guinea-pig 1 received 10 M.L.D. and 28 serum-units ¹ of Immune-serum and 3 c.c. per 100 grms. of <i>old</i> Rabbit serum	...	The animal died within 18 hrs.
Guinea-pig 2 received 10 M.L.D. and 28 serum-units of Immune-serum and 3 c.c. per 100 grms.	{ of <i>old</i> Rabbit serum, } { extract of fresh Ox clot }	recovered
Guinea-pig 3 received 10 M.L.D. and 28 serum-units of Immune-serum and 3 c.c. per 100 grms.	{ of <i>old</i> Pig serum, } { extract of fresh Pig clot }	recovered

That is to say the deficient addiment can be supplied not only by *fresh serum* as already shown, but also by an extract of *fresh clot*. This points distinctly to a close relation of addiment to the leucocytes and their products of disintegration which are the only bodies we at present know to be obtainable in quantity both from the clot and from the separated serum of shed blood. It follows therefore from the results of all the different observations quoted, that leucocytes contain addiment, and this not only in the living body but even *in vitro*, and that they yield it on requirement to a serum from which it was previously altogether absent. And that this addiment is a ferment *proper* to the leucocytes, and not one extracted by them from the plasma and stored for future use, is clear from the observations that exudates rapidly freed from their leucocytes contain no such ferment, though if the leucocytes remain its presence can be proved—a condition impossible if the addiment were not a veritable leucocytic ferment but one circulating freely in the blood-plasma to be taken up by cells in general according to their various requirements, as supposed by Ehrlich. It seems therefore also evident that the process which occurs in the peritoneal cavity, by which previously inactive antimicrobial serum becomes active, consists not in the modification under cellular influence of stable but inactive protective bodies into unstable but highly active agents, as maintained by Pfeiffer, but in the addition to that serum of the previously absent addiment.

In this connection I would further call attention to the increasing evidence that the Immune-body itself is also a leucocytic product. Thus Deutsch⁽⁸⁾ has shown that it is formed in the leucocytic tissues,

¹ The theoretical serum requirement.

and Bulloch proved more recently a very marked and definite relation in his haemolytic serum between the increase of the Immune-body, as well as the variations in its quantity during the process of immunisation, and the varying degree of leucocytosis which was present or had been produced in the animal immunised. Moreover the phenomena of natural immunity, in which the serum certainly contains no appreciable amount of Immune-body, come into harmony with the facts of the acquired variety, if it be admitted that the Immune-body-Receptors are pre-existent in the leucocytes. And this admission equally explains the intimate relation of the Immune-body to the addiment, which I have urged consists of leucocytic ferment.

Bacteriolytic addiments, as we have seen, rapidly undergo decomposition and disappear from Immune-serum. Those of haemolysis, on the other hand, apparently remain. This fact may probably be correlated with the great thermolability of the former as against the relatively thermostabile nature of the latter; and with the observation of Metschnikoff that, while in haemolytic action the macrophages are the chief performers, in bacteriolysis, on the contrary, phagocytosis is primarily and chiefly the concern of microphages. This points definitely to the source of origin for the addiment in the two cases being traceable to different varieties of leucocytes.

Increase of Addiment during Immunisation.

If addiment is a leucocytic ferment it would be expected that with the appearance of the leucocytosis which accompanies the artificial production of immunity there should appear an increase in the addiment-content of the blood. Yet von Dungern⁽⁹⁾ was unable to determine any such increase during the process of immunisation against red-blood-cells; and Bulloch⁽¹⁰⁾ in his recently published experiments comes to a similar conclusion. These observations however prove only that the addiment quantum of the separated serum is not markedly increased, and do not necessarily give an indication of the conditions in the living animal itself. Incidentally it may be pointed out also that while von Dungern considers that the addiment quantum of normal rabbits' serum, with which he compared that of the immune-sera of similar animals which had been immunised, is fairly constant, yet his results show that the volume of such serum required to complete a given definite reaction varied from $\frac{1}{40}$ c.c. to $\frac{1}{20}$ c.c., in other words, varied 100%. This would tend to show that the addiment content of serum

is an inconstant quantity, unless this variability prove to have been dependent on varying methods of preparation or durations of storage of von Dungern's sera. But, if the statement of the absence of an increase of the addiment in the serum by immunisation be accepted, as confirmed by Bulloch's observations, there still remains a simple explanation consonant with an actual increase of addiment in the living animal. For the amount of addiment found in the haemolytic serum depends not on the amount of addiment present in the blood—that is, on our view, upon its leucocytic richness—but solely on the amount set free into the serum after bleeding, that is, upon the number of leucocytes destroyed by the procedures and in the process of coagulation. And this is in no way necessarily affected by the leucocytic richness of the blood. It is therefore not to be expected that the serum should exhibit any remarkable increase in its addiment even though there were a marked increase of addimentary ferment in the blood.

But on the other hand direct experimental evidence can be obtained of the association of a definite increase of addiment with the establishment of resistance to infection. This may be sought in the phenomena observed when an excess of Immune-serum is given in cases of deficiency of addiment. For suppose a certain organism to possess two active phagocytes and that one new phagocyte can be produced on requirement at the expiration of an hour from the demand. Suppose also that the bacteria injected, multiplying if not destroyed, double in number every twenty minutes. Grant further that each phagocyte contains only sufficient Immune-body-receptors to enable its addiment to destroy two bacteria, but that a given quantity of Immune-serum when injected suffices to employ all the addiment of the phagocytes which then destroy a maximum of the bacteria apiece. Now inject into this organism twenty-one bacteria and the same dose of Immune-serum as will enable the destruction of twenty. It follows that twenty bacteria are destroyed, the Immune-serum injected is used up, and the phagocytes are exhausted. Of the bacteria one remains, and this multiplying as supposed is represented after the expiration of an hour by eight. There now appears a new-formed phagocyte whose addiment if supplied with Immune-body from without can destroy ten bacteria, but owing to the absence of a sufficiency of this body it actually destroys two only and the invasion continues to advance. If however additional Immune-body had been injected the infection would now have terminated in consequence of the timely formation of fresh addiment.

Hence by the exhibition of an amount of infective material greater than can be dealt with by the addiment content of the animal, together with an excess of Immune-serum above the theoretical requirement of the infecting dose, we can determine, by the resulting recovery or death of the animal concerned, the presence or absence of the formation of fresh addiment in the reaction to infection. Investigation along these lines was undertaken and experiments were made, starting from the dose of *B. typhosus* at which deficiency of addiment appears (namely in the present instance at 5 M.L.D.), and giving at the same time Immune-serum in excess. The following were the results obtained.

	Result
Guinea-pig 1 received 5 M.L.D. and the theoretical serum requirement ¹ viz. 13 serum-units (<i>control</i>)	died in 12-15 hrs.
Guinea-pig 2 received 5 M.L.D. and the theoretical serum requirement of 6 M.L.D. ² viz. 16 serum units	recovered
Guinea-pig 3 received 6 M.L.D. and the theoretical serum requirement of 8 M.L.D. ² viz. 22 serum units	recovered
Guinea-pig 4 received 10 M.L.D. and the theoretical serum requirement of 13 M.L.D. ² viz. 37 serum units	recovered

From this it appears that while animal 1 died from deficiency of addiment, animals 2, 3 and 4, in which there was equal or greater deficiency of addiment (in animal 4 much greater), were enabled by the sheltering effect of the serum given to gain time for the formation of fresh addiment which then with the excess of Immune-body supplied completed the destruction of the microbes. That is to say, fresh addiment can be formed in the animal under suitable conditions, and is so formed in the reaction to infection. And indeed the leucocytosis which admittedly occurs would be meaningless if the newly formed and active phagocytes were deficient in their essential ferments, as would follow if the theory of von Dungern were correct.

Fresh addiment being formed in the reaction to a single infection it must evidently be similarly formed throughout immunisation. If further evidence of such formation be required, it may be found in the consideration that since deficiency of addiment appears when a comparatively small multiple of the M.L.D. is given (in the experiments here quoted with 5 M.L.D.) it should be impossible, if new addiment is not so formed, to immunise an animal to withstand more than this number of M.L.D. And this is clearly not the case.

¹ Theoretical serum requirement of n M.L.D. is $\frac{nd-e}{d-e}$, cf. p. 89.

² Excess of serum; considerably more than the theoretical requirement.

In those instances, however, where, as has been mentioned, an animal may die although its fluids have been saturated with a great excess of Immune-serum it is evident that we have passed away from the case which has been just discussed to one in which the bacteria are so numerous and active as to be beyond the reach of any possible addiment-production by the animal. Moreover in these cases leucocytosis is inhibited and fails to appear, so that the natural formation of fresh addiment does not occur.

Relation of 'Agglutinins' to the Protective Substances.

Agglutination has been shown to bear a close relation to protection. In an endeavour to throw light on this relation I have made certain experiments with dead typhoid cultures.

The toxins of the *B. typhosus* are undoubtedly intracellular toxins, for even old cultures freed from bacilli by filtration possess no toxic action. The action of dead typhoid cultures is the action of these intrabacillary toxins. And Funck⁽¹¹⁾, who has worked upon this subject, states that his antityphoid serum had no greater antitoxic power against dead cultures than had a normal serum, though his figures might be thought to bear a different interpretation.

In the following experiments cultures were killed by heat, and a dose of the dead cultures was employed of nearly twice the M.L.D. of this material. This corresponded to the bacterial content of 20 M.L.D. of living culture, whose theoretical serum requirement was 58 serum-units. The control animal received a volume of normal serum equal to the volume of this number of units of Immune-serum. The results are given below.

	Result
Guinea-pig 1 received 20 M.L.D. living typhoid and 58 serum-units of Immune-serum	died in 16 hrs.
Guinea-pig 2 received 20 M.L.D. killed typhoid and 58 serum-units of Immune-serum	recovered
Guinea-pig 3 (control) received 20 M.L.D. killed typhoid and volume of normal Rabbit serum equal to volume of 58 serum-units ...	died in 14 hrs.

Accordingly the serum possessed *specific antitoxic action* not possessed by normal serum.

Now Gruber showed that if the agglutinative action of an Immune-serum be diminished by the application of heat, the protective power also undergoes a corresponding diminution. And this has been confirmed by Trumpp. I accordingly next heated a quantity of Immune-serum at 67° C. until its protective power against the living bacilli had been considerably reduced—as shown by direct trial and also by the fact

that its agglutinative action was diminished to about one-half its former value, namely from the limits

$$\begin{cases} 1 \text{ to } 550,000 + \\ 1 \text{ to } 600,000 - \end{cases}$$

to the limits

$$\begin{cases} 1 \text{ to } 300,000 + \\ 1 \text{ to } 350,000 - \end{cases}$$

it nevertheless still protected against the dead injections, though no longer against the living typhoid bacillus, even when given in excess of the theoretical requirement. Thus

	Result
Guinea-pig 1 received 2 M.L.D. living typhoid and 4 serum-units ¹ of the heated Immune-serum	died in 16 hrs.
Guinea-pig 2 received 2 M.L.D. living typhoid and 5 serum-units of the heated Immune-serum	died in 16 hrs.
Guinea-pig 3 received 20 M.L.D. killed typhoid and 58 ² serum-units of the heated Immune-serum	recovered

that is to say, the antitoxic power remained although the agglutinating action and the protective action against living cultures had been markedly diminished. The antitoxic action of the serum against the intracellular toxins, which can only come to act upon the animal infected after the solution of the micro-organisms themselves, has therefore no intimate relation with the agglutinative action which proceeds equally with the living as with dead bacteria.

Hence may be now deduced an explanation of the lack of complete parallelism between the phenomena of agglutination and protection. For the protection in the cases here considered must be a function of combined antimicrobial and antitoxic action, while the agglutinative process can be related only to the former of these two components. The parallelism therefore between agglutination and protection cannot be complete. It may be so between agglutination and bacteriolytic action.

Agglutination however is not concerned directly in the events of lysogenesis which is effected by the coordinated action of the Immune-body and addiment, and which can occur, and does with many microorganisms invariably take place, in the absence of agglutinative action. It must therefore be connected with the only other event with which we are acquainted in antimicrobial action, namely the phagocytic process. And I suggest that the "agglutinins" assist this process by their faculty of bringing the microorganisms together (and

¹ 4 serum-units is the theoretical serum requirement of 2 M.L.D.

² Theoretical requirement of 20 M.L.D. of living typhoid.

at rest if previously motile) in larger or smaller masses, and by the alteration in the bacterial envelope which they produce—changes which clearly simplify the process of ingestion.

CONCLUSIONS.

1. The amount of Immune-body needed for protection against n M.L.D. of a bacterium is contained in $\frac{nd - e}{d - e}$ c.c. of its Immune-serum, where d is the M.L.D. e the largest dose invariably not fatal and the serum-equivalent of one M.L.D.

2. Addiment is not extremely special to the species.
3. Addiment is a leucocytic ferment.
4. Addiment is increased during and by immunisation.
5. Agglutinins assist the phagocytic process of ingestion.

The experiments here brought forward were made in the Bacteriological Institute of the University of Berne during the early months of the present year. Many of them have since been repeated and confirmed either there or in the Pathological Laboratory of the University of Oxford. My best thanks are due both to Professor Tavel, of Berne, and to Dr James Ritchie, Reader in Pathology in the University of Oxford, for the facilities of their laboratories, and for their constant kindness and encouragement in the present work.

OXFORD, October 1901.

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RECENT RESEARCHES CONCERNING THE ETIOLOGY,
PROPAGATION, AND PREVENTION OF YELLOW
FEVER, BY THE UNITED STATES ARMY COM-
MISSION.

(Three Charts.)

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THE efficient control of the spread of yellow fever is a matter of such vast practical importance, both from the hygienic and commercial point of view—not only for the countries where this disease prevails as an epidemic, but also for those in which, after importation, it may assume epidemic proportions—that it has seemed appropriate to bring together in this paper a summary of the work thus far accomplished by the United States Army Commission¹ on the Island of Cuba, during the years 1900 and 1901, in order that English and Colonial readers who have not, perhaps, had access to the original contributions published in different American journals, may be able to form an intelligent opinion concerning the permanent value of this work. It will also afford opportunity for recording the more recent confirmatory observations made by others concerning the mode of transmission of yellow fever discovered by the Commission, and for calling attention to the results already obtained by the U.S. Army Medical Department in the suppression of this disease, especially in the city of Havana, through the enforcement of sanitary measures based on these later researches.

The American Commission was organized in May 1900, and began its investigations during the following month (June), being equipped with suitable laboratory facilities for practical work, both at the

¹ The members of this Commission were Major Walter Reed, Surgeon, U.S. Army, and Drs James Carroll, A. Agramonte, and the late Dr Jesse W. Lazear, Contract Surgeons, U.S. Army.

military garrison of Columbia Barracks, near Quemados, Cuba, and also in the city of Havana. As yellow fever was already prevailing at the time of our arrival in Cuba suitable material for the scientific study of this disease was immediately available.

The Etiology of Yellow Fever.

Before giving the results of our investigations it may be well to recall the situation as regards the etiology of yellow fever at that time. Briefly it may be said that the claims of all investigators for the discovery of the specific agent of yellow fever—since modern bacteriological methods had come into use—had been disproved by the exhaustive observations of Sternberg⁽¹⁾, published in 1890, except that made by Sanarelli⁽²⁾ for a small, motile bacillus isolated by him from the blood drawn during life in two of six cases of yellow fever, and from the blood and organs after death in seven of twelve cases of this disease (58 %), studied at Montevideo and Rio de Janeiro, Brazil. The results obtained, however, by those who had promptly undertaken to investigate Sanarelli's claim for the specific character of *Bacillus icteroides*, seemed to show a lack of agreement such as has never been reported, as far as the writer can recall, in connection with the supposed specific cause of any of the other acute infections. Thus while Achinard and Woodson⁽³⁾ had, during the epidemic of 1897 in New Orleans, La., isolated a bacillus, claimed by them to be identical with *B. icteroides*, from the venous blood in 4 out of 5 cases, and from yellow fever cadavers in 32 out of 39 cases (82 %), Portier⁽⁴⁾, working in the same city and during the same epidemic, could only obtain this bacillus 3 times in 51 autopsies, and failed to obtain it at all in cultures made from the venous blood during life in 10 cases. Again, while Wasdin and Geddings⁽⁵⁾, in the city of Havana, were able to cultivate *B. icteroides* from blood withdrawn from the lobe of the ear, "not earlier than the third day of the disease" in 13 of 14 cases (92·8 %), and to find it in 85·7 % of their necropsies, Agramonte⁽⁶⁾, studying the disease on the Island of Cuba, failed to isolate *B. icteroides* in a single instance from blood drawn from the lobe of the ear in 37 cases or from the blood drawn from a vein at the bend of the elbow in 31 cases, at various stages of the disease. The latter observer, however, reported finding this bacillus at autopsy in 11 of 35 cases (31·4 %). Without going further into detail, we may say that the results obtained by Lutz⁽⁷⁾ and

de Lacerda and Ramos⁽⁸⁾ in Brazil, and by Matienzo⁽⁹⁾ in Mexico, were equally conflicting and unsatisfactory.

Under these circumstances it seemed to the members of the Commission of the first importance to give their entire attention to the bacteriological study of the blood of those sick with yellow fever and of the blood and organs of yellow fever cadavers, having especially in view the isolation of *B. icteroides*. We were thus able during June, July, and August to take repeated cultures from the blood during life in 18 cases of yellow fever, adopting the usual method employed in withdrawing blood from a vein at the bend of the elbow, and transferring the blood, at once, in quantities of 0.5 c.c. to each of several tubes containing 10 c.c. of nutritive bouillon which were afterwards incubated at 37° C. for a period of one week. In seven cases, four of which were designated as "mild" yellow fever and three as "well-marked" yellow fever, only one culture was made from the blood in each case, viz.: in two cases on the 1st day; in one case on the 2nd day; in three cases on the 3rd day, and in one case on the 4th day. In the remaining eleven cases, diagnosed as "severe" yellow fever, of whom four died, more frequent cultures were taken from the blood, these varying from two to six cultures on as many different days of the disease. In two of the fatal cases, cultures were made each day from the commencement of the attack and including the day on which death occurred.

The negative result of these numerous cultures taken from the blood of cases of yellow fever, as regards the presence of *B. icteroides*, was reported in a "Preliminary Note" presented at the meeting of the American Public Health Association⁽¹⁰⁾, held in Indianapolis, Indiana, October 22nd—26th, 1900. To these eighteen cases we can now add six other cases, or a total of twenty-four, from which blood cultures have been made during life with negative results.

The importance of this negative finding as regards the growth of any specific bacterium will be better appreciated when it is seen, as I shall soon have occasion to point out, that yellow fever may be produced in non-immune human beings by the subcutaneous injection of a small quantity (0.5—2 c.c.) of blood withdrawn from the venous circulation of a patient suffering with this disease.

In addition to the results above recorded, the careful study of eleven autopsies was equally barren as to the presence of any particular micro-organism, although the quantity of material with which our tubes were inoculated was greater than is usually made use of at autopsies.

In a word, then, the careful bacteriological study which the Commission had made in cases of yellow fever had given no indications as to the presence of the specific agent of this disease. The same may be said concerning the result of numerous microscopic examinations of fresh and stained specimens of blood which we had in the meanwhile studied with the view of finding possibly some intracellular or extracellular body. Apparently no body, bacterial or protozoan, which could be brought into view with a $\frac{1}{12}$ Zeiss immersion objective, was present in the blood of these cases.

Although displaced from the order in which the following observations were made, it will be best to present, at this time, the results of the experiments which were later carried out by the Commission on non-immune human beings by means of the subcutaneous injection of blood, withdrawn during the active stage of the disease, as these results bear so directly upon the subject which we are now considering, viz. the etiology of yellow fever.

The only reference that I can find in the literature relative to an attempt to convey yellow fever in this way is cited by Sternberg⁽¹¹⁾, who states that at Vera Cruz, Mexico, in 1887, he saw Dr Ruis inject into a non-immune individual a hypodermic syringe of blood drawn from a case of yellow fever on the eighth day of the disease. The result was negative, as was also the result of two other attempts related to him by Ruis.

Our own observations, undertaken for the purpose of ascertaining whether an attack of yellow fever could be induced in a second individual by the injection of a small quantity of blood, embrace experiments made on twelve American soldiers and Spanish immigrants, all non-immune individuals.

These observations may be divided into the following classes :

1. Injection of the fresh blood taken from a vein at the bend of the elbow.
2. Injection of partially defibrinated blood.
3. Injection of partially defibrinated blood heated for ten minutes at 55° C.
4. Injection of blood-serum previously diluted with sterilized water and filtered slowly through a Berkefeld laboratory filter.

The following Table, I., gives the results of these several inoculations:

TABLE I.

No. of case	Quantity and material used	Day of disease	Date of inoculation	Result	Date of attack
I	2 c.c. fresh blood	Second	Dec. 26, 1900	Negative	
II	2 " "	"	Jan. 4, 1901	Positive	Jan. 8, 1901
III	1.5 " "	First	" 8 "	"	" 11 "
IV	0.5 " "	Second	" 22 "	"	" 24 "
V	1 " "	"	" 25 "	"	" 28 "
VI	0.75 c.c. partially defibrinated blood	Third	Oct. 15 "	"	Oct. 20 "
VII	1.5 c.c. partially defibrinated blood heated for 10 minutes at 55° C.	"	" 15 "	Negative	
VIII	Same as No. VII	"	" 15 "	"	
IX	" "	"	" 15 "	"	
X	1.5 c.c. of filtered blood serum	"	" 15 "	Positive	Oct. 19, 1901
XI	Same as No. X	"	" 15 "	"	" 19 "
XII	{ Same as No. X 2 c.c. fresh blood	{ Fourth	{ " 15 " " 22 "	{Negative {Positive	{ " 23 "

By an examination of this table it will be seen that of the seven individuals who received subcutaneously the fresh or partially defibrinated blood in quantities of 0.5—2 c.c., six (85.7%) developed an attack of yellow fever within the usual period of incubation of the disease.

These results are of very great interest as demonstrating that the *specific agent of yellow fever is present in the blood, at least during the first, second, and third days of the attack.*

Another important point brought out by these experiments was that *the blood which conveyed the disease did not contain any bacterium which would grow on our usual laboratory media.*

In order to establish this fact, as soon as blood had been injected into the non-immune subject, additional blood was, at once, withdrawn in considerable quantity and transferred to tubes of nutritive bouillon. In one instance, where 2 c.c. of blood had been drawn into the syringe, 0.5 c.c. of this sufficed, when injected, to produce a severe attack of yellow fever, after seventy-three hours' incubation, while the remaining 1.5 c.c. transferred immediately to four tubes of bouillon gave no growth, except that from one tube we isolated on the 4th day *Staphylococcus pyogenes citreus*, found by us to be a common skin-contaminating organism in Cuba.

Table I further shows that the specific agent contained in the blood is destroyed or attenuated by heating the latter at 55° C. for 10 minutes,

so that the injection of 1.5 c.c. of this heated blood was harmless (cases VII, VIII, and IX), while the injection of 0.75 c.c. of the same blood unheated sufficed to promptly induce an attack of yellow fever in a "control" individual (case VI).

Of not less interest was the fact brought out by these observations that yellow fever can be produced by the injection of a small quantity of bacteria-free serum filtrate, obtained by passing the diluted serum through a Berkefeld laboratory filter (cases X and XI), and further that the blood of a case of yellow fever, thus produced, when injected into a third non-immune subject will promptly bring about an attack of this disease (case XII); thus demonstrating that the specific agent of yellow fever can find its way through the pores of a filter which ordinarily serves to prevent the passage of all known bacteria.

I have elsewhere⁽¹²⁾ in conjunction with one of my colleagues (Carroll) discussed the facts here presented more at length and will limit myself, therefore, to the remark that these experiments appear to indicate that yellow fever, like the foot and mouth disease of cattle, is caused by a micro-organism so minute in size that it might be designated as ultra-microscopic.

The Propagation of Yellow Fever.

Prior to the time at which the foregoing observations were made the Commission had already turned its entire attention to the possible solution of the problem of the propagation of yellow fever, being induced thereto, not only by the fruitlessness of the investigations made thus far along bacteriological lines, but, also, by reason of certain facts which seemed to call for a better interpretation than had hitherto been accorded them.

Without entering into details, I may say that, in the first place, the Commission saw, with some surprise, what had so often been noted in the literature, that patients in all stages of yellow fever could be cared for by non-immune nurses without danger of contracting the disease. The non-contagious character of yellow fever was, therefore, hardly to be questioned.

In the second place, it had been observed that patients discharged from the wards during early convalescence could be brought into intimate association with non-immune individuals without thereby establishing fresh foci of the disease. This did not seem to indicate that any specific agent was present in the excreta of the sick.

Again, it had been noted that in certain cases of this disease no growth had been obtained on the ordinary laboratory media, either by frequent cultures from the blood during life or from the blood and organs after death.

Further, in the course of an investigation which the Commission were able to make during the last week of July, 1900, concerning the origin and spread of a small epidemic of yellow fever that had appeared in a military garrison, numbering about 900 men, at Pinar del Rio, Cuba, they had seen that by reason of the false diagnosis of "pernicious malarial fever" which had been given to these cases no disinfection of bedding or clothing had been carried out; and yet there was no indication that this neglect had contributed in the least to the spread of the disease; nor had any harm come to those non-immunes who had slept in the beds vacated by the sick, or washed the supposedly infected garments of those who had recovered or died of this disease.

Putting these various data together, it seemed probable that more progress might be made if attention should be turned to the mode of transmission of yellow fever, especially as our own observations had caused us to seriously doubt the usually accepted belief of the conveyance of this disease by means of *fomites*.

Then, too, the endemic curve of yellow fever in the city of Havana, and its well-known epidemic curve in the United States, appeared to be more intimately associated with and more affected by the rise and fall of the annual temperature curve than was to be seen in any of the acute infections, except malarial fever. The peculiar behaviour of this disease (if I may use the expression) in rapidly spreading in certain localities, when introduced, as contrasted with its failure to propagate itself in other places, where the conditions for its increase were apparently just as favourable, seemed to point in the strongest manner to the necessity for some special agent or intermediate host in the dissemination of its specific cause. If malarial fever—a disease so much affected by temperature conditions—required the agency of a special genus of mosquito for its propagation, as had in recent years been so brilliantly worked out by Ross, Grassi, Bastianelli, Bignami and others, it did not seem unreasonable to suppose that yellow fever—a disease so plainly controlled by seasonal conditions—might also depend on some such agent for its spread. Influenced by this line of reasoning, the Commission began, during the second week of August, 1900, its observations relative to the propagation of yellow fever by means of the bite of a certain species of mosquito—*Stegomyia fuscata*.

The work along this line was carried forward so rapidly that, within thirty days, eleven individuals had been bitten by infected *Stegomyia*, of whom two¹ developed well-marked attacks of yellow fever within the usual period of incubation, and under such circumstances as to positively exclude, in one case, any other possible source of infection.

Appreciating fully the importance of this discovery and in order to exclude all other possible sources of infection in our future observations, it was now determined to establish a Special Experimental Station where further observations could be made on non-immune human beings, both as to the propagation of yellow fever by means of the bite of the mosquito as well as by exposure to the most intimate contact with infected clothing and bedding, and this under the strictest enforcement of military quarantine. With the approval and assistance of the Military Governor of the Island of Cuba, this Experimental Station was ready for occupancy on November 20th, 1900, and was continuously occupied until March 1st, 1901.

As the results obtained at this station have already been published⁽¹³⁾ in full elsewhere, I will here only present a brief account, first of the experiments with fomites and afterwards of those made with infected mosquitoes.

Attempts at Infection by Fomites.

I quote from a paper which the writer presented for the Commission at the meeting of the Pan-American Medical Congress², held in Havana, Cuba, Feb. 4-7, 1901: "For this purpose there was erected at Camp Lazear a small frame house consisting of one room, 14 × 20 feet, and known as 'Building No. 1,' or the 'Infected Clothing and Bedding Building.' The cubic capacity of this house was 2800 feet. It was tightly sealed within with 'tongued and grooved' boards, and was well battened on the outside. It faced the south and was provided with two small windows, each 26 × 34 inches in size. These windows were both placed on the south side of the building, the purpose being to prevent, as much as possible, any thorough circulation of the air within the house. They were closed by permanent wire-screens of 0.5 mm. mesh. In addition a sliding glass sash was provided within and heavy wooden

¹ One of these cases was that of Dr James Carroll, Contract Surgeon, U.S.A., a member of the Commission.

² *Loc. cit.*

shutters without; the latter intended to prevent the entrance of sunlight into the building, as it was not deemed desirable that the disinfecting qualities of sunlight, direct or diffused, should at any time be exerted on the articles of clothing contained within this room. Entrance was effected through a small vestibule, 3×5 feet, also placed on the southern side of the house. This vestibule was protected without by a solid door and was divided in its middle by a wire-screen door, swung on spring hinges. The inner entrance was also closed by a second wire-screen door. In this way the passage of mosquitoes into this room was effectually excluded. During the day, and until after sunset, the house was kept securely closed, while by means of a suitable heating apparatus the temperature was raised to 92° — 95° F. Precaution was taken at the same time to maintain a sufficient humidity of the atmosphere. The average temperature of this house was thus kept up at 76.2° F. for a period of sixty-three days.

"Nov. 30, 1900, the building now being ready for occupancy, three large boxes filled with sheets, pillow-cases, blankets, etc., contaminated by contact with cases of yellow fever and their discharges were received and placed therein. The majority of the articles had been taken from the beds of patients sick with yellow fever at Las Animas Hospital, Havana, or at Columbia Barracks. Many of them had been purposely soiled with a liberal quantity of black vomit, urine, and fecal matter. A dirty 'comfortable' and a much soiled pair of blankets, removed from the bed of a patient sick with yellow fever in the town of Quemados were contained in one of these boxes. The same day, at 6 p.m., Dr R. P. Cooke, Acting Assistant Surgeon, U.S.A., and two privates of the Hospital Corps, all non-immune young Americans, entered this building and deliberately unpacked these boxes, which had been tightly closed and locked for a period of two weeks. They were careful at the same time to give each article a thorough handling and shaking, in order to disseminate through the air of the room the specific agent of yellow fever, if contained in these fomites. These soiled sheets, pillow-cases and blankets were used in preparing the beds in which the members of the Hospital Corps slept. Various soiled articles were hung around the room and placed about the bed occupied by Dr Cooke.

"From this date until Dec. 19, 1900, a period of twenty days, this room was occupied each night by these three non-immunes. Each morning the various soiled articles were carefully repacked in the aforesaid boxes, and at night again unpacked and distributed about the

room. During the day the residents of this house were permitted to occupy a tent pitched in the immediate vicinity, but were kept in strict quarantine.

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“December 19th these three non-immunes were placed in quarantine for five days and then given the liberty of the camp. All had remained in perfect health, notwithstanding their stay of twenty nights amid such unwholesome surroundings.

“During the week December 20—27 the following articles were also placed in this house, viz. pajamas suits, 1; under-shirts, 2; night-shirts, 4; pillow-slips, 4; sheets, 6; blankets, 5; pillows, 2; mattresses, 1. These articles had been removed from the persons and beds of four patients sick with yellow fever and were very much soiled, as any change of clothing or bed-linen during their attacks had been purposely avoided, the object being to obtain articles as thoroughly contaminated as possible.

“From Dec. 21, 1900, till Jan. 10, 1901, this building was again occupied by two non-immune young Americans, under the same conditions as the preceding occupants, except that these men *slept every night in the very garments worn by yellow fever patients throughout their entire attacks*, besides making use exclusively of their much-soiled pillow-slips, sheets and blankets. At the end of twenty-one nights of such intimate contact with these *fomites*, they also went into quarantine, from which they were released five days later in perfect health.

“From January 11 till January 31, a period of twenty days, ‘Building No. 1’ continued to be occupied by two other non-immune Americans, who, like those who preceded them, have slept every night in the beds formerly occupied by yellow fever patients and in the night-shirts used by these patients throughout the attack without change. In addition during the last fourteen nights of their occupancy of this house they had slept each night, with their pillows covered with towels that had been thoroughly soiled with the blood drawn from both the general and capillary circulation, on the first day of the disease, in the case of a well-marked attack of yellow fever. Notwithstanding this trying ordeal these men have continued to remain in perfect health.

“The attempt which we have therefore made to infect ‘Building No. 1’ and its seven non-immune occupants, during a period of sixty-three nights, has proved an absolute failure.”

Infection by Mosquitoes.

While the experiments with *fomites* were being carried out in "Building No. 1," certain non-immune individuals who were lodged in tents, in a separate part of the camp, were being subjected, with their full consent, to the bites of mosquitoes which had previously fed on the blood of cases of yellow fever occurring in the city of Havana. Thus during the period from December 5th, 1900, to February 7th, 1901, we had subjected to this method of infection twelve non-immune subjects, who had previously passed their full record of quarantine in this camp. Of these 10, or 83.3%, experienced attacks of yellow fever and always within the period of incubation of this disease.

The following Table II. gives the necessary data concerning these observations:

TABLE II.

No. of case	Days in quarantine	Inoculation		Method of inoculation	Period of incubation in hours	Result	Order of occurrence	Date of occurrence
		Hour	Date					
I	15	2 p.m.	Dec. 5, 1900	Mosquito	81½	Positive	I	Dec. 8, 1900
II	9	4 p.m.	" 8 "	"	137	"	III	" 13 "
III	19	10.30 a.m.	" 9 "	"	83½	"	II	" 12 "
IV	21	4.30 p.m.	" 11 "	"	91½	"	IV	" 15 "
V	32	12 noon	" 21 "	"	95	"	V	" 25 "
VI	31	10 a.m.	Jan. 8, 1901	"	—	Negative	VI	—
VII	22	11 a.m.	Dec. 30, 1900	"	94½	Positive	VII	Jan. 3, 1901
VIII	69	8.30 p.m.	Jan. 19, 1901	"	95½	"	VIII	" 23 "
IX	74	10.30 a.m.	" 25 "	"	—	Negative	IX	—
X	6	9.30 a.m.	" 31 "	"	74½	Positive	X	Feb. 3, 1901
XI	78	11 a.m.	Feb. 6 "	"	78	"	XI	" 9 "
XII	25	2 p.m.	" 7 "	"	70	"	XII	" 10 "

The positive results obtained, therefore, by this mode of infection stand in striking contrast to the negative experiments made with *fomites*. Indeed, cases VIII and XI of Table II had each slept twenty-one nights in the garments of yellow fever patients while occupants of Building No. 1. As they had remained in perfect health at Camp Lazear for yet thirty days longer, they were at the expiration of this time bitten by infected mosquitoes solely for the purpose of testing their immunity and with the result that an attack of yellow fever promptly followed in each case.

It should be borne in mind, also, that of the non-immune residents at Camp Lazear, while all lived under the same hygienic conditions,

only those individuals developed yellow fever who were purposely bitten by contaminated mosquitoes, or injected with the blood of those sick with this disease. Moreover, the precision with which the infection of the individual followed the bite of the mosquito left nothing to be desired in order to fulfil the requirements of a scientific experiment.

Case V of Table II is of especial interest, when taken in connection with the failure to induce the disease by contact with *fomites*.

This individual, having been quarantined for thirty-two days at Camp Lazear, volunteered to enter a newly erected building in which fifteen contaminated mosquitoes had just been freed. His first visit was at noon, December 21, 1901, and the length of his stay thirty minutes. At 4.30 p.m. the same day he again entered this building and remained twenty minutes. The following day at 4.30 p.m. he, for the third time, visited this room and remained twenty minutes. During each of these visits he was bitten by mosquitoes. He did not enter the building again, nor was he exposed to any other source of infection. Nevertheless at the expiration of three days and twenty-three hours, or at 6 a.m. December 25, 1900, he was suddenly seized with an attack of yellow fever, which proved to be severe in character. That the infection was occasioned by the bites of contaminated mosquitoes was plainly shown by the immunity from the disease enjoyed by two non-immunes "controls," who, protected only by a wire-screen partition, had been present at each of the subject's visits and who, under the same conditions of security against the bites of the infected mosquitoes, continued to sleep in, and breathe the common atmosphere of this room for yet eighteen nights.

To the positive cases contained in Table II, which were produced at Camp Lazear, we are now able to add four other cases of yellow fever occasioned by the bites of infected mosquitoes, thus making a total of fourteen cases, in each of which happily recovery followed.

A very important point brought out by these observations is that an interval of about twelve days or more after contamination appears to be necessary before the infected *Stegomyia* is capable of conveying the disease to a susceptible individual. Repeated experiments made with insects which had bitten yellow fever patients two to ten days previously were always negative, although these same insects were proven capable of conveying the disease after having been kept until 17 to 24 days had elapsed. Our observations⁽⁴⁾ further demonstrate that mosquitoes that have been kept for periods varying from 39 to 57 days after contamination are still capable of conveying the disease, and

further that infected *Stegomyia* may survive for a period of at least 71 days. This will explain how the contagion of yellow fever may cling to a building, although it has been vacated for a period of two or more months.

Bearing in mind that the observations made by means of blood injections (Table I) were only undertaken *after* we had succeeded in demonstrating that the disease could be conveyed by the bites of infected *Stegomyia*, it will be seen that our study of the method of propagation of yellow fever, at Camp Lazear, sufficed to prove very definitely that, while the natural mode of transmission of this disease is through the bites of infected mosquitoes, yellow fever may also be conveyed, like malarial fever, by the injection of a small quantity of blood taken from the veins of an individual suffering with this disease.

Per contra, our observations show that, notwithstanding the common belief in this mode of transmission, yellow fever cannot be induced in the non-immune individual even by the most intimate contact with contaminated articles of clothing and bedding.

Although the investigations made at Camp Lazear were only concluded one year ago, already confirmatory evidence of the strongest character has been furnished in a series of experiments carried out by Guit  ras⁽¹⁵⁾ at the Inoculation Station of the Sanitary Department of Havana.

I may be pardoned for quoting the paragraph with which Guit  ras begins his contribution. He says: "The favourable results obtained by the United States Army Commission in their experiments with yellow fever, the continued series of mild cases resulting from these experiments without a death, suggested very naturally the continuation of their work on a larger scale; not with a view to control or confirm the conclusions of the Commission, for anyone who had followed their work with unprejudiced attention must have concluded that their solution of the problem of the etiology of yellow fever was final; but rather in the hope of propagating the disease in a controllable form, and securing amongst the recently arrived immigrants immunization, with the minimum amount of danger to themselves and the community."

Of a total of 42 individuals inoculated by Guit  ras 25 were rejected by him by reason of having been bitten by insects that had been applied to cases of fever about which the diagnosis was in doubt. The following table, therefore, only includes 17 persons who were bitten by *Stegomyia* that had previously fed on unmistakable cases of yellow

fever at intervals of 14 to 36 days before being applied to the non-immune subject.

TABLE III.

No. of case	Date of inoculation	Mode of inoculation	Result	Period of incubation
1	Feb. 23, 1901	Mosquito	Positive	3 days, 10 hours
2	Aug. 4, 1901	"	Negative	
3	" 4 "	"	"	
4	" 7 "	"	"	
5	" 8 "	"	Positive	4 days, 5 hours
6	" 8 "	"	"	3 " 3 "
7	" 7 "	"	Negative	
8	" 9 "	"	Positive	5 " 3 "
9	" 10 "	"	Negative	
10	" 10 "	"	"	
11	" 10 "	"	"	
12	" 13 "	"	Positive	3 days, 19 hours
13	" 13 "	"	Negative	
14	" 14 "	"	Positive	3 " 21 "
15	" 14 "	"	"	5 " 21 "
16	" 22 "	"	"	3 days
17	" 24 "	"	Negative	

A more complete confirmation of the results obtained by the American Commission could not be furnished than the data contained in the foregoing table, since they show that of 17 individuals who were bitten by infected *Stegomyia fasciata*, eight (47%) developed the disease. Most unfortunately in three of these cases very grave symptoms ensued, such as black vomit and suppression of the urine, which eventuated in the death of the patients. I may add that in the hands of Guitéras *fomites* failed to exert any effect on non-immunes.

Whether other species of mosquitoes than *Stegomyia* are capable of conveying the parasite of yellow fever has not as yet been determined by the Commission: nor have we been able to ascertain whether the parasite passes from the mother insect to daughter insects. The experiments which we have thus far been able to make for the purpose of determining these important points, although negative, have been too few in number to warrant any definite expression of opinion.

The Prevention of Yellow Fever.

The definite determination of the way in which yellow fever is transmitted from the sick to the well, furnishes a solution at last of that much vexed problem of how to prevent the spread of the disease.

Even in the absence of more definite knowledge concerning its specific agent—knowledge greatly to be desired from the scientific standpoint—we are now able, as sanitarians, to direct our efforts along certain well-defined lines, with a feeling of security heretofore unknown.

From the point of view of prevention the situation may be briefly summed up in the following conclusion, which was presented by the American Army Commission to the Pan-American Congress of 1900¹, viz. "The spread of yellow fever can be most effectually controlled by measures directed to the destruction of mosquitoes and the protection of the sick against the bites of these insects."

This conclusion was the logical outcome of the observations that had been made by the Commission at its Experimental Station near Quemados, Cuba.

The importance of the discovery that yellow fever is transmitted by the bite of a certain species of mosquito did not fail to attract the prompt attention of the Military Governor of the Island of Cuba, himself a physician and formerly a distinguished member of the Medical Department of the United States Army. By his direction the theory was at once subjected to a practical test in the city of Havana, in which city yellow fever had not failed to make its yearly appearance during the past one hundred and forty years.

Under the efficient management of the Chief Sanitary Officer, Surgeon-Major Wm. C. Gorgas, U.S. Army, the sanitary regulations were so far modified as to require that every patient having yellow fever should not only be quarantined, but that his room should be promptly protected with wire-screens, so as to prevent the possibility of mosquitoes becoming infected by sucking the blood of the patient. As a second important measure, a systematic destruction of all mosquitoes in other rooms of the patient's house, as well as in adjoining houses, was at once begun, the fumes of *pyrethrum* being relied upon to stupefy the insects, after which they were carefully swept up and burned. In other words, Surgeon-Major Gorgas relying upon the well-known slow progress of yellow fever sought to destroy all mosquitoes, infected or non-infected, within a given radius of each case, while at the same time he effectually excluded all mosquitoes from access to the sick. If a secondary case occurred, the same hygienic measures were vigorously enforced along the lines above indicated.

As an illustration of what has been accomplished by these newer

¹ *Loc. cit.*

CHART I.

Showing monthly mortality from Yellow Fever in the City of Havana, for the twenty years, 1880—1899, and for the years 1900—1901.

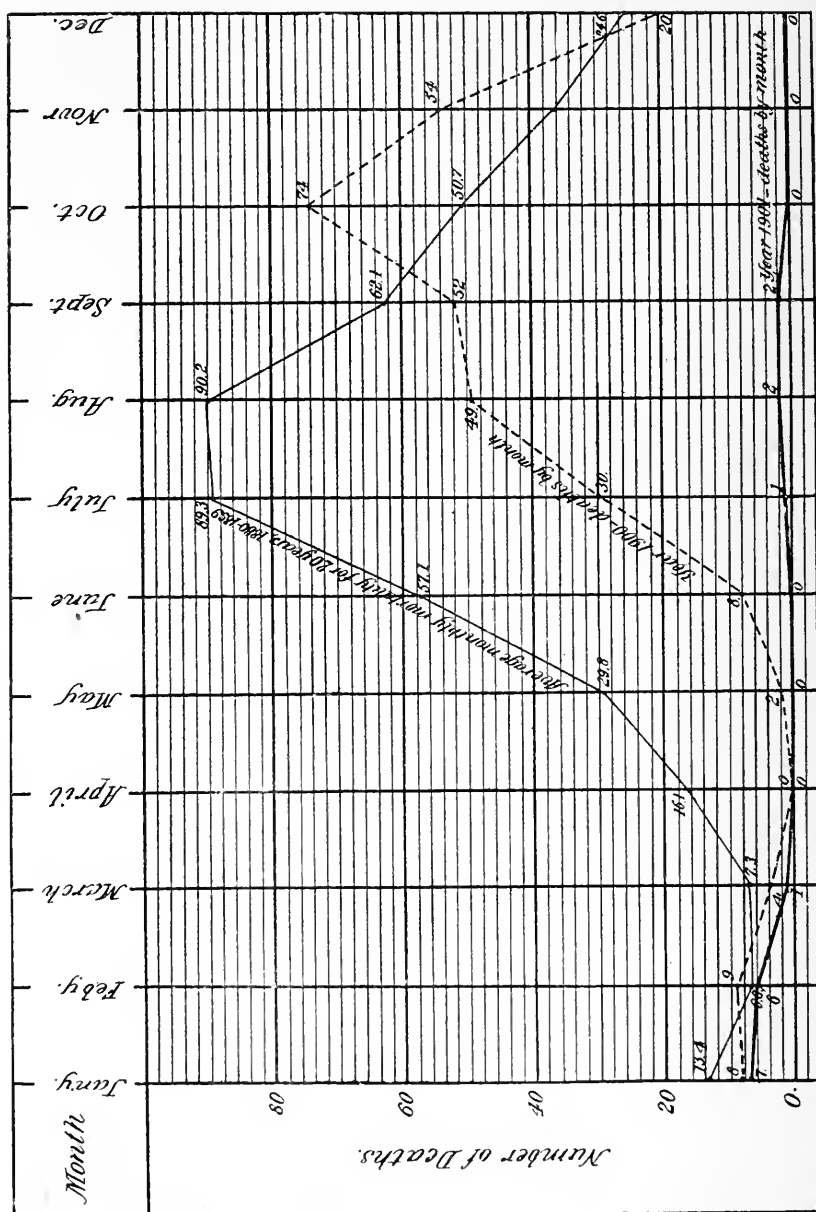


CHART II.

Cases and Deaths from Yellow Fever in the City of Havana, for the Epidemic year, March 1, 1900, to March 1, 1901 (by month).

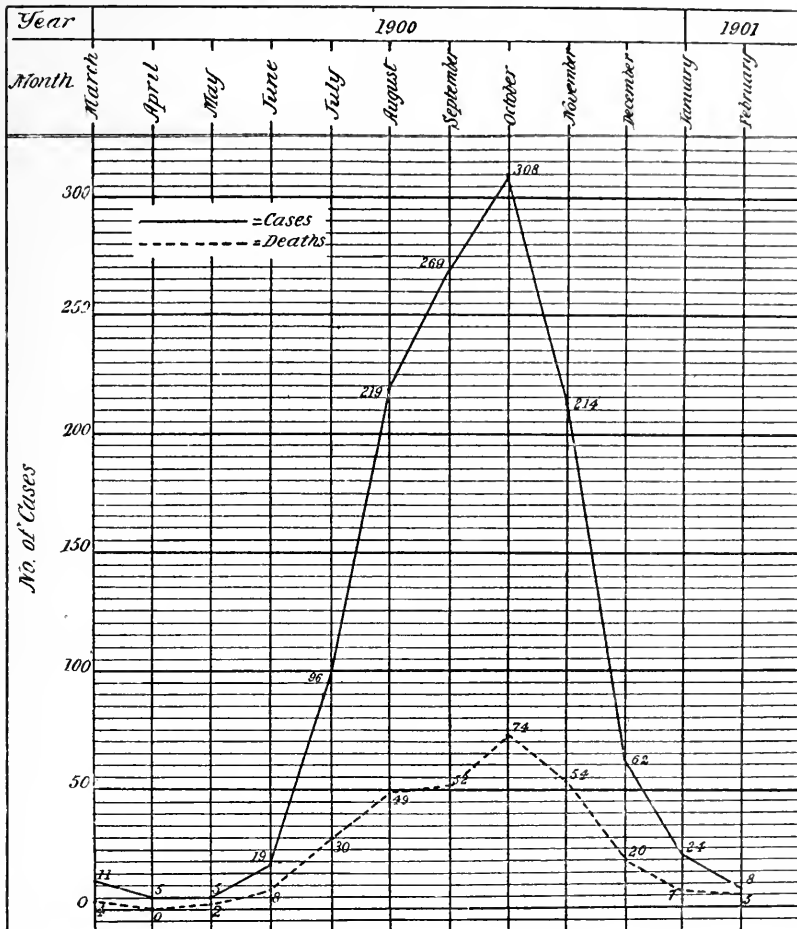
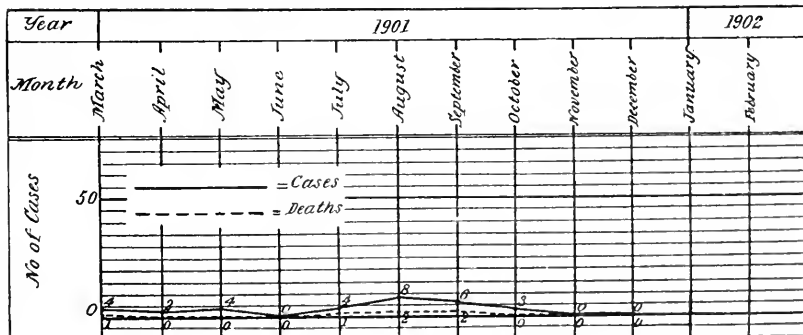


CHART III.

Cases and Deaths from Yellow Fever in the City of Havana, for the Epidemic year, March 1, 1901, to March 1, 1902 (by month).



sanitary regulations, I may state that counting from the date when they were put into force, viz. February 15, 1901—Havana was freed from yellow fever within ninety days; so that from May 7th to July 1st—a period of fifty-four days—no cases occurred. Notwithstanding the fact that on the latter date and during the months of July, August, and September, the disease was repeatedly reintroduced into Havana from an inland town, no difficulty was encountered in promptly stamping it out by the same measures of sanitation intelligently applied both in the city of Havana as well as in the town of Santiago de las Vegas, whence the disease was being brought into Havana.

As a further illustration of the remarkable sanitary victory accomplished over a disease whose progress we had heretofore been powerless to arrest, I will close this paper by inviting the reader's attention, first to the accompanying Chart I, which shows the average monthly mortality from yellow fever in Havana for the twenty years, 1880—1899, inclusive, and also the mortality by month for the years 1900 and 1901. I will then ask him to examine Chart II, which shows the progress of yellow fever in Havana during the epidemic year, ending March 1, 1901, when the sanitary authorities were putting forth every effort known at that time to sanitary science in order to control the march of the disease; and when he has satisfied himself that no effect whatever was produced upon the epidemic of that year, I will invite his attention to Chart III, which shows the occurrence of this disease in Havana for the epidemic year March 1st, 1901, to March 1st, 1902, during which year yellow fever was fought on the theory that the specific agent of this disease is transmitted solely by means of the bites of infected mosquitoes. By carefully comparing the figures both as to deaths and cases in these two Charts, and recalling that between the years 1853 and 1900 there have been recorded in the city of Havana 35,952 deaths from yellow fever, he will then be able to more clearly appreciate the value of the work accomplished by the American Army Commission.

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A NEW ACID-FAST STREPTOTHRIX, PATHOGENIC TO MAN AND ANIMALS.

(Plate I: Two Figures.)

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THE numerous additions to the group of *Streptothrices* which have been recently made, and the close resemblance which the majority of these newly described forms bear to one another, render it by no means an easy task to assure oneself that a freshly isolated member of this group is one that has not previously been met with and described. A curious fact also, which soon strikes anyone who investigates the literature relating to this group of micro-organisms, is that in very few of these has the particular form described by one author been met with by another. While great interest to the bacteriologist naturally attaches to any new species, whatever its source, the group of those which are pathogenic in their action on man, on animals, or on both, are, *ipso facto*, of more general importance. To this last category belongs the new member of the group which we have isolated from a fatal case of lung disease and pericarditis in man.

While the general morphological resemblance between the numerous forms of *Streptothrix* described is sufficiently clear to allow of no doubt as to their belonging to the same family, the points in which they differ from one another are less marked. These points may be shortly classified into (a) Pathogenicity to man or animals. (b) Cultural characteristics. (c) Staining reactions.

The organism under discussion was detected during life by means

of the last of these criteria, so the staining properties may be referred to in the first instance. In the smaller manuals of Bacteriology as a rule only two organisms, the *Bacillus leprae* and the *Smegma bacillus*, are noted as having the same peculiarity as the *Bacillus tuberculosis* in resisting decolorisation by a 25 per cent. solution of mineral acids after having been stained in hot carbol-fuchsin. This property is shortly designated as "Acid-fast" by German writers and is not uncommonly possessed by the various species of *Streptothrix*. To this acid-fast group the *Streptothrix* we have isolated belongs, and much of the interest at first attaching to it was due to the fact that, in the case from which it was isolated, the clinical diagnosis of pulmonary tuberculosis was thought to be confirmed owing to the appearance in the sputum of acid-fast rods, in many instances closely resembling *B. tuberculosis*. More careful examination however showed points of difference which will be detailed below.

Our knowledge of acid-fast microbes has of late been greatly extended. Nocard (1888) found that the organism causing 'Farcin du Boeuf,' the *Streptothrix bovis*, stained in this manner. The *Streptothrix* isolated by Eppinger (1890), from a diseased condition in man, presented the same characteristic, as did that described by Sabrazès and Rivière (1895). The organisms described by Petri and Rabinowitsch (1897), and the three forms found by Moeller (1897), viz. his 'Mist' Bacillus and Grass Bacilli Nos. 1 and 2, also resisted decolorisation by acids, but have not been found as the cause of disease in man. Pappenheim (1898) has recorded a case of gangrenous lung abscess in which bacilli were found, closely resembling *B. tuberculosis*, but, apparently, he did not succeed in obtaining a culture of the organism. The second organism described by Berestnew (1898) as a Pseudo-Actinomyces, which produced a fatal illness in a shoemaker, was also acid-fast. He also states that, in six cases of actinomycosis in cattle, the clubs in the younger nodules stained well by the method used for *B. tuberculosis*.

The history of the case from which the new *Streptothrix* was isolated was as follows :—

F. E. 26 years, Private 5th Dragoon Guards, belonged to the beleaguered garrison of Ladysmith. He contracted a fever there in January 1900, complicated with dysentery, from which he never completely recovered. On his arrival at Netley in May, 1900, he was evidently dangerously ill, pale, emaciated and suffering from hectic fever. Signs of fluid were found in his right pleural cavity and great enlargement of the liver. He had cough, with expectoration of reddish muco-purulent

sputum. On microscopical examination of the sputum numerous acid-fast rods were found, closely resembling *B. tuberculosis*. At the same time a few thin, segmented branching filaments, also acid-fast, were noted and supposed at the time to be the actinomycotic form of *B. tuberculosis*. There was no infiltration of the skin of the thorax. An exploratory puncture was made in the right pleural cavity and some odourless pus of a chocolate colour and somewhat slimy consistence was removed. Cultures made from this pus yielded the organism in question in nearly pure culture. The resemblance of the pus to that derived from a liver abscess was so striking that it was considered to have possibly arisen from that organ. On the following day Major Dick, R.A.M.C., made two incisions, one in the right posterior axillary line in the 7th interspace, the other in the right nipple line in the 8th interspace, and evacuated a quantity of pus, of the character described above. There was no marked haemorrhage nor discharge of soft brain-like matter, such as has been described in most cases of actinomycosis (Godlee, 1900). The liver was punctured to determine whether the empyema was due to the bursting of a hepatic abscess, with a negative result. No improvement of the general condition followed on the operation. Signs of pericarditis appeared, and the diarrhoea and fever increased and were accompanied by increasing prostration. He died 11 days later on the 21st May, 1900.

At the *post mortem* examination the right pleural cavity was found to be the seat of an advanced empyema. Both layers of the pleura were greatly thickened and coated with a rough pyogenic membrane. Both operation wounds communicated freely with the pleural cavity. The right lung, which was firmly adherent to the diaphragm, was in a condition of chronic broncho-pneumonia, but was free from nodules or cavities. Its weight, combined with that of the liver, with which it was removed 'en masse,' was 7 lb. 10 oz. (3458 grms.). The left lung weighed 2 lbs. (907 grms.) and was studded throughout with small cirrhotic nodules, showing no trace of caseation and differing in many respects from miliary tubercles. The liver was greatly enlarged and somewhat pale on section. On its convex surface it showed a small ecchymosed track, penetrating 1 inch into the right lobe—the side of the exploratory puncture noted above. There were neither fresh abscesses nor cicatrices of old ones. The heart, which, when freed from clot, weighed 12 oz. (340 grms.) showed some hypertrophy of the left ventricle but was otherwise normal. The pericardium was greatly distended, 22 oz. (625 c.cm.) of serum containing numerous flakes of lymph being removed from it. Both layers were thickened and coated with a shaggy deposit of recent lymph.

There was some thickening and pigmentation of the mucous surface of the colon in the neighbourhood of the sigmoid flexure, and a few shallow ulcers with irregular margins.

Cultures were made from the nodules in the left lung, the empyema pus, and from the pericardial fluid, with the result that the *Streptothrix* was again recovered, in pure culture, from the pericardial fluid and also, in conjunction with pyogenic organisms, from the pus and the lung.

Microscopically the *Streptothrix* was seen in scrapings from the pneumonic nodules in the lungs in large quantity, and presented the same characters as were noted in the sputum during life, except that the branching was more luxuriant. Sections of hardened lung tissue showed a chronic pneumonic process with numerous cirrhotic foci and the presence in considerable numbers of acid-fast bodies closely resembling those figured by d'Arrigo. None of the usual appearances of tubercular disease, giant cells, epithelioid cells, or caseation, were found.

The *Streptothrix* occurred in the pus as a fairly open network of long thin threads, segmented and showing lateral branches which came off at right angles. In length, they were seen occasionally to stretch almost across the field of the microscope ($\frac{1}{12}$ " oil immersion). The width of the threads averaged about 0.5μ and was fairly uniform throughout without any appearance of clubs or spore formation. The threads stained well with all basic aniline dyes and retained Gram's stain. When treated by the Ziehl-Neelsen Carbol-Fuchsin and decolorised by 25 per cent. sulphuric acid and alcohol they remained deeply stained.

Behaviour in Cultures.

Very slight growth, if any, occurred at room temperature. On *Gelatine Plates*, after 5—7 days' incubation at 22°C ., small white circular colonies appeared on or close to the surface. On examination by a low power these were seen to resemble small hemispherical balls of cotton-wool, snow-white by reflected light and centrally tinged yellowish-brown by transmitted light. No liquefaction of the medium occurred after three weeks' incubation, and the colonies ceased to enlarge after 8—10 days, attaining a maximum diameter of about 1 mm. In *Gelatine-stab* cultures a growth appeared on the surface similar to that on plates with a slight expansion for a short distance down the needle track.

On *Agar* its growth is rapid. After 36—48 hours a snow-white dry powdery growth appears which a few days later takes on a delicate pale coral-pink tinge where the growth is thickest. The pink tint is very

constant and appears, after a varying period, on cultures in nearly all media where the organism is freely exposed to the air. The water of condensation remains clear. A few rugged granules may appear on the thicker portion of the agar slope, but they show no tendency to run together into a film, and attain a maximum diameter of about 1 mm. in a week. The growth is, in cultures of less than 10 days' incubation, easily removable by the needle from the surface of the agar, and, even in old cultures, never adheres as firmly to the medium as do so many other forms of *Streptothrix*. (See Plate I, fig. 1.)

In ordinary nutrient *Broth*, after 24—36 hours' incubation at 37° C., delicate white specks are seen floating on the surface, and this appearance, likened by Besson to water-lily leaves, is common to the majority of the *Streptothrices*. These specks increase slowly in size and become more or less spherical in shape, the portion above the surface of the fluid being dry and tinged coral-pink, while that below appears woolly and white. The growth, while never forming a felted, coherent scum, may extend up the sides of the tube, but, as a rule, the separate rosettes or colonies, after 1—2 weeks, sink to the bottom of the tube, retaining their shape and individuality. The broth throughout remains clear and odourless and its alkaline reaction unaltered. No indol is produced. The addition of a little sterile synovial fluid to ordinary broth enhanced the growth of the organism and increased its microscopical resemblance to the form in which it was originally noted in the empyema pus.

On *Potato*, which, whether glycerinated or not, forms a very favourable medium, a copious dry white growth occurs in 48 hours, giving the appearance of a splash of plaster of Paris. The growth never extends more than 2—3 mm. from the needle track, and though becoming granular and warty with age shows no tendency to wrinkle on the surface. The coral-pink tint develops early and is noticeable on the 3rd or 4th day, especially when the superficial white powdery coating is removed by the needle.

In *Milk*, a surface growth of isolated rosettes occurs, similar to that in broth. No clotting occurs, but in old cultures the milk is digested and a deposit of rosettes and casein takes place. The reaction of the milk is alkaline.

Solidified blood serum. No growth.

In Sterile Tap-water. After four days a somewhat scanty growth appeared on the surface resembling that in broth, but the floating colonies sank to the bottom of the fluid in a shorter time.

Conditions of Growth.

While free development occurs at 22° C. and optimum growth at 37° C., temperatures much higher than this have failed to devitalize it; indeed, it might almost be included in the group of thermophilic bacteria. Growth took place on potato after 48 hours at 46.5° C. but none after incubation at 50° C. Heating for five minutes at a temperature of 75° C. failed to destroy it, though its subsequent cultivation was much delayed. Twenty minutes exposure to 75° C. or momentary exposure to 100° C. destroyed it.

Under conditions of anaerobiosis by displacement of Oxygen by Hydrogen or CO₂ no development took place.

It retains its vitality for a long time. A dried-up agar culture, nine months old, was successfully transplanted.

Morphology of the Streptothrix in Cultures.

While careful examinations and comparison of the character of growth in the various culture-media disclosed numerous slight differences, it will suffice to indicate the features common to them all, only indicating the chief variations. (See Plate I, fig. 2.)

The *Streptothrix* occurs, like most of the members of the group, in two forms, a fine branching network of mycelial threads, and a so-called 'streptococcic' form caused by the breaking up of the terminal threads into a series of small oval segments, whose exact nature has not yet been determined. These latter may, for purposes of description, be termed arthrospores but, though they differ from true spores in their origin, being clearly due to a segmentation of the endo-capsular contents of the original thread, it appears to us probable that they function as true spores and that it is to their abundant formation under suitable conditions that the longevity of the organism is due.

Briefly, on dry media, the threads rapidly break up into arthrospores and these form the dry white powder which overlies the mass of the culture. On the other hand, in fluid media, while the aerial portion of the floating island is largely composed of these arthrospores, the portion below the surface of the fluid consists chiefly of the network of branching threads in which, although segmentation of the threads may be observed, the further development of the segments into arthrospores is but rarely seen.

The acid-fast nature of these two forms of the organism varies with the age of the culture. Klein and Moeller have noted similar features

in old cultures of Tubercle and Grass Bacillus II respectively. When young, both threads and arthrospores retain the fuchsin intensely, later, by degrees, the threads lose this property and, in old cultures, they may become completely decolorised. On the other hand the arthrospores remain acid-fast for as long as we have had them under observation. The same remark applies to Gram's staining, the threads with age becoming decolorised while the arthrospores retain the stain. After growing for some months in the medium to which sterile synovial fluid had been added, dark, almost black beads were noted in the length of the threads on staining by Weigert's method, similar to those arthrospores of the tubercle bacillus figured by Coppen Jones (1895), these beads occasionally were found at the point at which a lateral branch was given off from the main thread. Many stages of the segmentation of threads into arthrospores have been noted in cultures from different media and of different age, from a chain of rods resembling a small chain of *B. anthracis* to a perfect streptococcal form, in some of which a trace of the membrane binding them together could still be detected. The arthrospores when fully developed and free are of fairly uniform size and oval in shape, but do not, when unstained, possess the high refrangibility of bacterial spores.

All forms of the *Streptothrix* are non-mobile, and nothing approaching clubs or involution forms has been noted even in old cultures.

Pathogenicity.

Intraperitoneal inoculation of guinea-pigs caused their death in from five to six weeks. Large collections of caseous matter were found, matting together the omentum and small intestine. Metastatic deposits of like nature were found behind the peritoneum, in the diaphragm and in the testes. When inoculated hypodermically under the skin of the thigh of guinea-pigs, a similar collection of caseous matter formed, breaking down into an ulcer which gradually healed. The presence of the organism, chiefly in the form of threads, was in each case observed in this caseous matter, while in the case of the peritoneal collection the *Streptothrix* was recovered in pure culture.

Comparison of the above cultural and other characteristics of this organism with the published accounts of those of the great majority of other *Streptothrices* will show at once wide differences. There are, however, others in which the resemblance is closer, and it may be well to refer briefly to these, indicating the points of difference between them and the new *Streptothrix*.

The *Streptothrix* isolated by Eppinger (1890) from a brain abscess, while not unlike morphologically, differs from ours by growing to some extent anaerobically, by its characteristic growth in broth—dense surface layers of feltwork which sink to the bottom and are renewed again and again—, and also by the orange tint which all its cultures take on. A culture of this form obtained from Král's laboratory, while growing well on ordinary media, refused to grow in sterile water.

The *Streptothrix* of Sabrazès and Rivière (1895) grew only anaerobically and is thus excluded.

Berestnew's 2nd *Pseudo-Actinomyces* (1890) grew under anaerobic conditions. Gelatine and potato inoculated with it remained sterile. Broth cultures had a foetid smell. It was not pathogenic to guinea-pigs and survived but a few generations.

Nocard's *Streptothrix bovis* (1888) forms a dry membrane on agar and a scaly curdy layer on potato. It liquefies gelatine slowly and shows no apparent growth in milk.

Moeller's Mist Bacillus and his Grass Bacillus 1, or 'Timothy Grass Bacillus' are closer allied to *B. tuberculosis* than to ours. They produce clouding in broth and a bright yellow or orange colour in the various media.

Moeller's Grass Bacillus 2 produces a soft and moist yellow growth on agar and renders milk acid.

The *Streptothrix* isolated by Dean (1900) was not acid-fast and did not grow on potato or gelatine.

The forms recorded by Buchholtz (1897) and Flexner (1898) as occurring in man presented many points of resemblance to that under discussion, but in neither case were they successful in cultivating the organism.

DESCRIPTION OF PLATE.

PLATE I.

Fig. I. Photograph of a pure culture of the *Streptothrix* on agar, after one week's incubation at 37° C.

The individual colonies will be noted to be fairly uniform in size and shape, and to show no tendency to coalesce or to wrinkle on the surface. The colonies are snow-white unless touched with a needle, in which case the characteristic coral pink colour will be found underlying the superficial layer of white 'dust,' formed by the arthrospores.

Fig. II. Film preparation of the *Streptothrix*, from a pure culture in peptone broth, one month old. Stained in warm carbol-fuchsin and treated with 25 per cent.

sulphuric acid and absolute alcohol. Microphotograph made with Zeiss Apochr. 2 mm. N. A. 1.4 and P. Oc. 3 Magnification, 1000 diameters.

The Streptothrix threads, showing lateral ramifications and various stages of segmentation, retain the stain with varying degrees of intensity. The arthrospores, uniformly "acid-fast," are seen in various aggregations, from the streptococcal forms, produced by direct segmentation of one of the threads of the Streptothrix, to the free and isolated arthrospores, nearly uniform in size and oval in shape.

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FIGURE 1.



FIGURE 2.

AN EXPERIMENTAL ENQUIRY ON THE DISINFECTION OF FLOORS FOR PLAGUE.

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THIS investigation has been carried out under the orders of the Government of Bengal on the lines suggested by the Plague Commission in Chapter VI., paragraph 83, of their report, namely, by determining what strengths of the different disinfectants are required to be used in order to kill all the species of micro-organisms in the floor which are not considerably more resistant than the plague bacillus itself. Under these circumstances, any plague bacilli would be pretty certain to be destroyed, and this indirect method of ascertaining the strengths required for the purpose is necessitated by the impossibility of isolating plague bacilli from infected floors preventing direct experiment.

As not only the best but also the cheapest efficient disinfectant is required, some guide to the most likely ones to experiment with can be obtained by the simple method of ascertaining the strength required to kill the plague bacillus in test-tubes, as their effectiveness under these conditions is likely to afford some approximate guide to their relative power under more complicated conditions, and also allows of the elimination of those whose cost must evidently be prohibitive. The summary of previous observations given in paragraph 69 of the Plague Commission's report is of great value here, and they have been supplemented and extended in the experiments now under report. The strengths of various disinfectants required to kill the plague bacillus in from 10 to 15 minutes are summarized in the following table, which embodies the results of previous observers as well as my own.

Table of minimum strengths which kill plague bacilli in 10 to 15 minutes in test-tubes.

Disinfectant	Previous workers' results	The writer's results
Perchloride of mercury	1 in 5000 to 1 in 10,000	1 in 10,000
Perchloride of mercury dissolved in weak HCl	—	1 in 20,000
Permanganate of potash	1 in 10,000	1 in 10,000
Phenol	1 in 400 to 1 in 1000	1 in 750
Sulphuric acid	1 in 2000	1 in 1000
Hydrochloric acid	1 in 500	1 in 1500
Nitric acid	1 in 333	1 in 1200
Lysol	1 in 400	—
Formalin	—	1 in 750
Chloride of lime	1 in 100	—

NOTE.—In all my experiments the time-limit was 15 minutes, which accounts for the somewhat higher figures under some heads, as other workers have sometimes adopted a limit of only 10 minutes.

Taking the figures of the above table as a guide to the relative strengths of the disinfectants dealt with, and working out the cost of effective solutions of each, it was found that formalin and lysol were prohibitively expensive. Further, chlorinated lime is unsuitable, both on account of the cost of adequate strengths and its instability and varying composition. On the other hand, perchloride of mercury, phenol, permanganate of potash and the mineral acids are all sufficiently cheap to be worthy of careful examination.

The method suggested by the Plague Commission has been closely followed: only plates were found to be more efficient than agar slopes for the purpose of isolating the different organisms which survived the disinfection, in order to examine further their resisting powers; and consequently they were always used. As in most of the disinfections in towns, and nearly all those in villages, mud or rammed earth floors have to be dealt with, which present much greater difficulties than do paved floors, it was determined to test what strength of the different disinfectants were necessary for the effective treatment of mud floors, as such strengths would be still more reliable on impermeable floors, and hence would meet all requirements. A room with a mud floor, which had been leeped¹ with cowdung some weeks before, and had recently

¹ The process of leeping consists in mixing cowdung with water until it forms a paste, the latter being applied to the floor. At times mud, or a little chopped up straw or dried grass, is added to the paste. When dried the floor presents a fairly smooth surface, which however does not last long because of the formation of cracks and the breaking away of dried flakes, consequently the process of leeping has to be repeated every week or two. The paste is also applied after the manner of plaster to walls and ceilings. We are indebted to Captain H. Gidney, I.M.S., for this information.—G.H.F.N.

been inhabited by a sweeper, was selected for the experiments, and it proved to have a sufficiently varied bacterial flora to be admirably adapted for the work. Areas about two square feet in extent were nearly surrounded by low mud walls, and flooded with half a litre of the solutions, which were then allowed to run off. As soon as the surface had dried, which usually took between one and two hours, plugs of sterilised cotton-wool were rubbed over it and pieces with the adherent earth sown in broth. After incubation, plates were made, and the different species of colonies isolated in pure cultures. The resisting power of these to the same disinfectant previously used was then tested by exposing a loop-full of agar culture to different strengths of the disinfectant in test-tubes for 15 minutes, and then inoculating from them into broth, and noting in which the organisms grew. By comparing these results with those obtained in a similar manner with cultures of the plague bacillus, the resisting powers could be compared. If any organisms which had little or no greater resisting power than the plague bacillus itself were obtained from the floor after its disinfection, it was obvious that the strength used was not a safe or effective one. If, on the other hand, all the organisms isolated had much greater power of resistance than the plague bacillus, all weak ones having been destroyed, then the disinfectant might be relied on to kill the plague bacillus under similar circumstances.

EXPERIMENTS.

I. *Mineral acids*.—These have a powerful action on the plague bacillus, while they are also cheap. On the other hand, their action will be weakened by being partly neutralized by the alkalies present in the earth and cowdung of Indian floors, as is shown by the great effervescence which occurs on applying them. They will also have a deleterious effect on certain kinds of stone and cement with accompanying neutralization. They have been tested in strengths of 1 in 200, or from five to ten times that found necessary in test-tube experiments, with the following results:—

Sulphuric acid 1 in 200—500 were poured over two square feet of floor. Three different micro-organisms were subsequently obtained from the floor and severally exposed to different strengths of the acid for 15 minutes, and then inoculated into broth. One of them was killed by a 1 in 800 solution, but survived after exposure to a 1 in

1,000 one, being thus only slightly more resistant than the plague bacillus, while another survived in a 1 in 1,200 solution, but was killed by a 1 in 1,000 strength, being thus as feebly resistant as the plague bacillus itself. Sulphuric acid in a 1 in 800 solution, therefore, is not a reliable disinfectant of mud floors.

Hydrochloric acid 1 in 200 was tested in a similar manner to the above. Four different organisms subsequently cultivated from the floor were further tested in different strengths of this acid. One was highly resistant, being only killed by a 1 in 200 solution, surviving exposure to one of 1 in 300 strength. The other three were killed by a 1 in 1,000 solution, only surviving a 1 in 1,200 one, thus being very little more resistant than the plague bacillus. Hydrochloric acid in a strength of 1 in 200 is, therefore, not a reliable disinfectant of mud floors.

Nitric acid 1 in 200 was also tested as above, and two organisms isolated from the floor after its application were further tested as to their resisting powers in various strengths of this acid. Both were killed in 15 minutes by a 1 in 1,000 strength, only surviving a 1 in 1,200 solution, being thus scarcely more resistant than the plague bacillus. A 1 in 200 solution of nitric acid cannot, therefore, be relied on to kill the plague bacillus in a mud floor.

From the above experiments it appears that the mineral acids in strengths from five to ten times as great as those required to destroy the vitality of the plague bacillus in test-tube experiments are yet inoperative against organisms nearly or quite as weak as the plague bacillus when they are applied to mud floors. This is doubtless due to the rapidity with which they are neutralized when spread over mud floors. Stronger solutions would be too destructive for use as disinfectants on a large scale, so it is evident that these chemicals are not suitable for use by themselves as disinfectants against plague.

II. *Permanganate of potash* has a very powerful action on the plague bacillus in a test-tube, but is liable to be decomposed by the organic matter contained in a mud floor of an inhabited room. It has been tested in strengths of 1 in 1,000 and 1 in 500, or in quantities from ten to twenty times that which is effective against the plague bacillus in test-tube experiments, with the following results:—Two organisms were isolated from the mud floor after the use of a 1 in 1,000 solution. One of them was highly resistant, but the other, a *Staphylococcus*, was killed by a 1 in 6,000 solution of permanganate, and only resisted exposure for 15 minutes to a 1 in 10,000 solution,

being thus scarcely more resistant than the plague bacillus. A 1 in 1,000 solution of permanganate, therefore, cannot be relied on to destroy the plague bacillus in a mud floor. A 1 in 500 solution was therefore tested in the same way as before. A variety of organisms were isolated from the floor after its use, some of which were highly resistant sporogenic bacilli. Three other organisms were tested with the following results:—A short non-sporogenic bacillus was killed by a 1 in 1,200 solution, but survived 15 minutes in a 1 in 1,400 one. A small Coccus survived a 1 in 1,000 solution. Both germs were considerably more resistant than the plague bacillus. A third, a Staphylococcus, was destroyed by one of 1 in 2,000 strength, but survived a 1 in 5,000 one, being somewhat more resistant than the plague bacillus. As the number of colonies obtained after the disinfection from the floor was also very considerable, this experiment was not altogether satisfactory evidence of the efficiency of the solution used, so the same strength was tried again, a different portion of the floor being used as in the other experiments. Again, a number of colonies appeared in the plates, including a sporogenic bacillus and two species of Cocci, and on testing them, each resisted the action of a 1 in 500 solution of the permanganate for 15 minutes, being thus much more resistant than the plague organism, the result in this case being satisfactory. As the above two experiments did not give quite parallel results, the same strength solution was used once more, and yet again a number of organisms survived the process. On testing their resisting powers, no less than three different species of micro-organisms were found to be killed in 15 minutes by a 1 in 10,000 solution of permanganate of potash, being thus as feebly resistant as the plague bacillus itself. This failure, together with the large number of organisms which survived in each previous trial, shows that a 1 in 500 solution of this salt is not to be relied on as a disinfectant against plague in the case of mud floors. As this strength is twenty times as great as will kill the plague bacillus in test-tubes in 15 minutes, it is evident that the organic matter in the floor must have largely neutralized the permanganate solution; and as the amount of organic matter in different mud floors must vary greatly, it is evident that no reliance can be placed on this salt in solutions which would not be so strong as to be too costly, while the deleterious effect of organic matter on the solution would introduce an undesirable element of uncertainty which makes this chemical an unsuitable one for the purpose for which it is required.

III. *Phenol*.—This very cheap disinfectant was found to destroy the vitality of *Bacillus pestis* in test-tubes in 15 minutes in a strength of 1 in 750, while it survived exposure for the same time in a 1 in 1,000 solution. It was, therefore, tested in strengths of 1 in 100 and 1 in 50 in the same manner as before. After flooding with a 1 in 100 solution, a variety of organisms were isolated from the floor, some of which were found to have very little more resisting power than the plague bacillus, so that this strength was manifestly too weak to be effective against plague. On the other hand, a solution of 1 in 50 gave much more satisfactory results, as the following data show: in the first only three different organisms were obtained from the floor after disinfection, a Coccus and two sporogenic bacilli, all of which resisted exposure for 15 minutes to a 1 in 50 solution of phenol. In the second experiment five different organisms were recovered from the floor after disinfection. One was a highly resistant sporogenic bacillus frequently seen in this course of observations. The other four were further tested, and all of them survived 15 minutes in a 1 in 50 solution of phenol.

In both the last experiments phenol in a strength of 1 in 50 destroyed all the organisms in the floor which were not much more highly resistant than the plague bacillus, so that this strength proved to be an efficient disinfectant of mud floors, and *à priori* of impermeable ones.

IV. *Perchloride of mercury*.—This is the disinfectant which has been most extensively used in operations against plague in India; and as it has also been provisionally recommended by the Plague Commission, dissolved in dilute hydrochloric acid and in a strength of 1 in 1,000, it has been carefully tested. In the first place a 1 in 1,000 solution of the salt in water without the addition of any acid was used, and after the disinfection, three organisms were isolated from the mud floor. One of these was a spore-forming bacillus, which was only destroyed within 15 minutes by a 1 in 1,000 solution, while it survived a 1 in 1,200 one, being thus very highly resistant; the second was destroyed by a 1 in 2,000, but resisted a 1 in 4,000 one, being highly resistant; while the third only survived a 1 in 10,000 solution, being scarcely more resistant than the plague bacillus itself. A solution of corrosive sublimate of a strength of 1 part in 1,000 of water cannot, then, be relied on to kill the plague bacillus on the surface of a mud floor, no doubt on account of the precipitation of the salt under these conditions in an inert combination.

Acid perchloride of mercury.—Next, a trial was made of one part of the perchloride and two parts of strong hydrochloric acid in 1,000 of water, this being the solution which has been so extensively used in Calcutta during the last two or more years. This combination, I have found, kills the plague bacillus in test-tubes, when used in a strength of one part perchloride in 20,000 of water, the acid being in double the proportion of the salt as just mentioned. The 1 in 1,000 solution might reasonably have been expected to prove an efficient disinfectant, even of mud floors, against the plague bacillus, yet the four following experiments prove that it is not so. In the first trial three different organisms were isolated from the floor after disinfection, two of which were highly resistant sporogenic bacilli, which were only destroyed by solutions of the strength of 1 in 1,000 and 1 in 2,000, respectively, of perchloride in acid solution. The third, a *Staphylococcus*, was killed by a 1 in 4,000 solution, only resisting a 1 in 6,000 one, and thus being not very markedly more resistant than the plague bacillus. In the second experiment, in addition to two highly resistant sporogenic bacilli, two different *Micrococci* were isolated from the floor after disinfection. On testing them both were found to be destroyed by a 1 in 6,000 solution in 15 minutes, only resisting the action of a solution of 1 in 10,000, being thus very little more resistant than the plague bacillus. Thus in the first experiment the margin of safety was a small one, while in the second the disinfectant completely failed to destroy *Micrococci* of very little greater resisting power than the plague bacillus. The acid perchloride of mercury solution in a strength of 1 in 1,000 is therefore not a reliable disinfectant for use on mud floors, so that the failure of the extensive disinfection operations in Calcutta bustees last year can be easily understood.

As the failure of the above solution to disinfect mud floors appeared to be very possibly due to the precipitation of the mercury salt by the alkalis in the soil, owing to the amount of acid present being too small, it was resolved to try the same strength of perchloride, namely, 1 in 1,000, but with double the usual amount of hydrochloric acid, namely, four parts per 1,000, or 1 in 250, which in itself is an active agent in destroying the plague bacillus in test-tube experiments. Two experiments were carried out with this solution with the following results:—In the first trial three different organisms were recovered from the floor after the disinfection, one of which was a highly resistant sporogenic bacillus, frequently met with in these experiments. Another was a *Coccus*, which survived a 1 in 1,000 solution, being thus highly

resistant. The third was a Coccus, which was destroyed by a 1 in 6,000 solution, only resisting a 1 in 10,000 solution; being thus but little more resistant than the plague organism. In the second experiment, five different organisms survived the disinfecting process, and no less than two of these were subsequently found to be destroyed in 15 minutes by a 1 in 10,000 solution, being thus no more resistant than the plague bacillus itself, so that the latter, if it had been present, would in all probability have also survived the process, and the strength used was practically useless.

This result is a surprising one, for it shows that organisms may survive quite on the surface of a mud floor which has been flooded with a disinfectant mercurial solution in an acid medium 20 times stronger than that required to destroy the same organism in 15 minutes in a test-tube. That the organisms isolated from the floor were really present in it before the application of the disinfectant is certain, for the precaution was taken in this and in other experiments of covering up the patch with a large bell-jar immediately after the disinfectant had been poured on, and it was only removed at the time the sample of earth was taken within an hour or two, when the patch was dry, the room having been shut up and not visited in the meantime. Further, the organisms tested had also been isolated from undisinfected parts of the same floor, and the same species were repeatedly met with in different experiments. It is clear, then, that a 1 in 1,000 solution of perchloride of mercury, even with double the usual strength of hydrochloric acid, is not an efficient or reliable disinfectant for use in the case of mud floors, so that the strength so extensively used in various parts of India during the last few years is not sufficiently strong for village and bustee disinfections, although, as will be seen immediately, it is effective in the case of impermeable floors.

Experiments were next carried out with double the usual strength of acid perchloride, namely, one part of the mercury salt and two parts of hydrochloric acid in 500 of water instead of in 1,000, with the following results:—In the first trial no colonies were obtained from agar surface cultures inoculated from the broth tubes in which the disinfected earth had been sown, and on making plates only one very highly resisting sporogenic bacillus, which resisted exposure for 15 minutes to a 1 in 1,000 solution, was obtained. This was a highly satisfactory result. In a second experiment the same resistant sporogenic bacillus was obtained, together with a Coccus, which was considerably more resistant than the plague bacillus, also a good result. The very

small number of colonies isolated after the use of this 1 in 500 acid perchloride solution was as marked a feature of its use as the resisting power of those which survived, it equalling the 1 in 50 phenol solution in the latter respect, while it was superior to it in the former one. In the case of the particular floor used for these experiments, then, the 1 in 500 acid perchloride of mercury solution proved to be an efficient disinfectant for destroying organisms of as little resisting power as the plague bacillus, although in the ordinarily used strength of 1 in 1000 it proved to be unreliable.

DISINFECTION OF IMPERMEABLE FLOORS.

In large towns a certain number of houses with paved floors may have to be disinfected, although Dr Hossack, who has had a large experience in the most crowded areas of Calcutta, informs me that plague cases comparatively rarely occur in such houses, if the floors are in good repair and clean, as in the best Bengali houses. On the other hand, in the filthy Marwari houses plague is very common. In view, however, of the great strength of the solutions found necessary for the efficient disinfection of mud floors, it seemed to be advisable to try if somewhat more dilute ones might not be sufficient for impermeable floors. Phenol and corrosive sublimate solution were used for this purpose.

Four experiments were carried out with a 1 in 1,000 solution of perchloride of mercury in weak hydrochloric acid as recommended by the Plague Commission, with the following results:—In the first two experiments the amount of solution used was only just enough to flood the floor area used, which was composed of flag stones. Of four plates made from four broth tubes sown with material from the disinfected surface as soon as it was dry, one proved to be quite sterile, and three only yielded a very few colonies. Staphylococci were isolated and further tested, but in each case they were found to require a strength of 1 in 2,000 acid perchloride solution to kill them in 15 minutes, so that they were at least ten times as resistant as the plague bacillus. In the remaining two experiments a slightly larger amount of the same solution was used, and the floors were found to have been completely sterilised. As the floor areas used formed a part of the small animal house, it was anything but sterile before disinfection. These results were highly satisfactory, and a 1 in 1,000 acid perchloride of mercury

solution may be relied on for the disinfection of impermeable floors against plague.

Four experiments were also carried out in a similar way on other portions of the same floor with a 1 in 100 solution of phenol, this being twice as dilute as was found necessary for the disinfection of mud floors. In three instances the number of colonies was small after the disinfection of the paved floor, while in the fourth it was somewhat larger. In each case (in addition to a highly resisting sporogenic bacillus in two instances) Staphylococci were separated, but all of them proved to be highly resistant forms, as they all survived exposure for 15 minutes to a 1 in 100 solution of phenol, being, therefore, much more resistant than the plague bacillus. It appears, then, that a 1 in 100 solution of phenol is an efficient disinfectant of impermeable floors against the plague bacillus, although it is not quite so powerful against the more highly resistant forms of micro-organisms as a 1 in 1,000 acid perchloride of mercury solution is. As with both chemicals some Cocci survived, it would not be advisable to try weaker solutions than these.

SUMMARY AND CONCLUSIONS.

The above experiments show that neither the mineral acids nor permanganate of potash are reliable disinfectants of mud floors against the plague bacillus, even when used in solutions which are many times as strong as are required to destroy the plague bacillus in test-tubes in 15 minutes. This is doubtless owing to the former being neutralized by the alkalies contained in the earth and in the cowdung used for leeping, and to the latter being precipitated and rendered inert by combining with organic matter, which is plentifully present in the leeped mud floors of rooms inhabited by the poorer classes in the large towns and villages of India, which are so often affected by plague.

Of the disinfectants which are sufficiently cheap to be employed on a large scale, there remain phenol and perchloride of mercury in acid solution, the former of which has given satisfactory results on mud floors in a strength of 1 in 50, while the latter is effective in a strength of one part of the mercury salt and two parts of strong hydrochloric acid in 500 of water. If we compare these strengths with those respectively required to destroy plague bacilli in 15 minutes in test-tubes, we find that 1 in 750 of phenol and 1 in 20,000 of acid perchloride of mercury solutions are effective under the latter cir-

cumstances. Thus it appears that in order to be sure of killing micro-organisms of as little resisting power as the plague bacillus on the surface of mud floors, 15 times as strong a solution of phenol and 40 times as strong a one of acid perchloride of mercury as are effective against the plague bacillus in test-tubes in 15 minutes must be used. Thus in proportion to its action on the plague bacillus in test-tubes, phenol is nearly three times as effective on a mud floor as is acid perchloride of mercury. This fact clearly indicates that a portion of the mercury salt is rendered inert by combining with certain constituents of the mud floor, probably albuminous substances, and this may explain the at first sight somewhat surprising failure of a 1 in 1,000 mercurial solution efficiently to disinfect the mud floor. However, when the strength of this solution was doubled, the acid being also doubled, satisfactory results were obtained with the mud floor tested, and the micro-organisms recovered after its use proved to be much more highly resistant than the plague bacillus, while they were fewer than even after the use of 1 in 50 phenol. Nevertheless, the neutralizing action of mud floors on the mercury solution will doubtless vary considerably in different places, and it is easy to conceive that in some instances this variable and unrecognisable factor may be considerably greater than in the case of the particular floor used in these experiments, and that even a 1 in 500 solution might be ineffectual under such circumstances. On the other hand, phenol appears to be free from this objection; and as it gave as good results in a strength of 1 in 50 as the acid perchloride did in one of 1 in 500, it must be considered the safer disinfectant in this strength (1 in 50) in the case of mud floors. In the case of impermeable floors either a 1 in 100 solution of phenol or a 1 in 1,000 one of acid perchloride of mercury is efficient, the latter being somewhat the more powerful of the two, which advantage is probably more than counterbalanced in practice by the disadvantage of using two different disinfectants, if the conclusion that phenol is the better in the case of mud floors is accepted. Both 1 in 50 phenol and 1 in 500 acid perchloride of mercury cost about one penny a gallon, the former being very slightly the cheaper of the two in such price-lists as I have been able to consult.

The variable effects of mud floors in partially neutralizing the disinfectant power of perchloride of mercury solutions may possibly account for the different estimations of the value of disinfection with this agent in different places and provinces, and also for the failure of

the very extensive operations of the year 1900 to prevent the recurrence of plague in the same houses and bustees during the recrudescence of the disease in Calcutta early in 1901. It must also be remembered that the above-noted strengths of disinfectants only act on the superficial layer of floors, etc., and have no influence on the presence of rats, by which, it is now pretty generally admitted, plague is in some indirect way spread—an important factor which must not be lost sight of in estimating the probable advantages of measures of disinfection. Still it is doubtless a good thing to destroy any plague bacilli lurking in a room which has been inhabited by a plague patient, and the practical outcome of the present enquiry is that the perchloride of mercury in hydrochloric acid must be used in at least double the strength that was provisionally recommended by the Plague Commission, and which has up to now, I believe, been very generally relied on in Calcutta. It is still better to substitute a 1 in 50 solution of phenol in every case when mud or earth floors or courtyards form any part of the area to be dealt with, as will be the case in the great majority of instances in which disinfection for plague is required.

OBSERVATIONS ON THE MOVEMENTS OF THE POLLUTIONS OF THE TYNE ESTUARY DURING THE SUMMER OF 1901.

(Two Figures.)

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THE present absence of any Statutory regulations as regards the discharge of sewage and other pollutions into tidal waters has allowed many estuaries in this country to be converted into receptacles for the filth of the sea-port and manufacturing towns situated near the mouth of the larger rivers. Only by an order of the Local Government Board, made after local enquiry and on sanitary grounds, can an estuary be declared a stream and thus brought under the various Rivers Pollutions Acts.

There is good reason to believe that in certain estuaries the polluted water is carried up and down by the tide and is cleared out to sea comparatively slowly. Dibdin (Report to the London County Council, No. 227, 1894) has demonstrated in the Thames the existence of a long zone of such pollution. In parts of this zone the dissolved oxygen at times falls to 0.6 c.c. per litre, or about one-tenth the normal amount—the oxygen being taken up by the micro-organisms for the oxidation of the organic matter present in the water.

Parry and Adeney have this year communicated to the Institution of Civil Engineers the results of their investigations of the condition of the estuary of the Liffey after the discharge into it of the sewage of Dublin. So far these are the only studies of the kind with which I am acquainted.

During the summer of 1901, while studying the influence of pollutions upon fish, I made a series of observations on the estuary of the Tyne, which is of interest in connection with the question of the movements of the polluted waters in that estuary.

In these investigations the amount of dissolved oxygen was taken as the measure of the degree of pollution, since the extent to which the dissolved oxygen is removed from the water depends upon the amount of organic matter undergoing putrefactive or bacterial disintegration in it. From the fishery point of view, it was the only question of interest, since it had been shown that the prejudicial action of such polluted water upon fish is due to the diminution in the oxygen required for respiration and not to the presence of toxic substances.

Method.

Ramsay's method of estimating the dissolved oxygen was adopted, since it is rapid and gives results sufficiently accurate for the present purpose. (*Journal of Chemical Industry*, 30th Nov. 1901.)

It was impossible to carry out these analyses at the river side, and all samples had to be sent by train to Edinburgh. It was therefore essential that some method should be adopted which would give concordant and comparable results. Dibdin's plan of shaking the water up with air to full saturation and then leaving it exposed to air for 24 hours appeared to me the fairest means of measuring the probable condition of the water in the river channel, since the fully oxygenated water of the non-tidal part of the stream is in the estuary exposed to air from which it can take up oxygen.

It was found that with most samples of water an equilibrium was established between the water and the air, so that the amount of oxygen in solution remained the same for considerable periods at the laboratory temperature of about 13° C. The following table, showing the number of c.c. of dissolved oxygen per litre of water, after different periods, illustrates this statement.

Samples of September 4 (Low Tide).

No. of sample	Days after shaking		
	2	4	6
1	1	—	1
2	1	1	1
3	5	3	—
4	2	2	2
5	1	—	2
6	3	—	2

Samples of September 4 (High Tide).

No. of sample	Days after shaking			
	2	4	6	13
1	3	—	2	3
2	3	3	2	
3	3	2		
4	4	2	2	
5	5	—	5	
6	5	5	5	

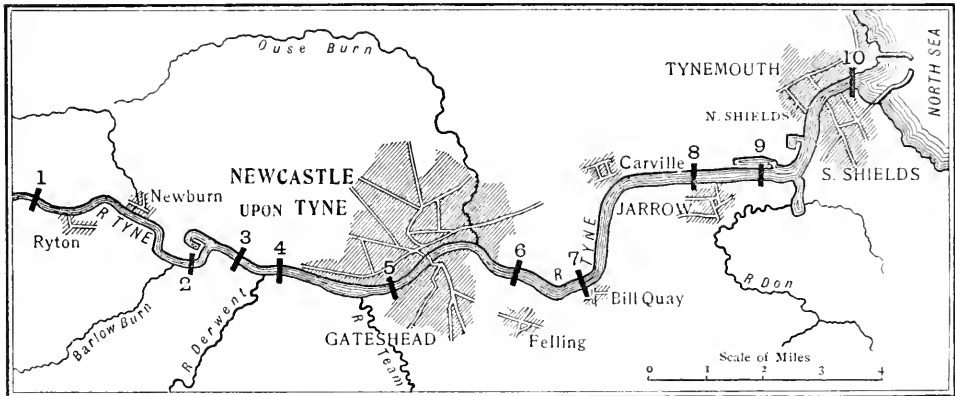
Samples of July 19th.

Nos. of samples	Days after shaking		
	1	2	3
5 & 6	5—5	4—4	4—4
2 & 3	2.5—2.5	3—3	3—3

To show the extent to which sea-water was present the chlorine of the various samples was determined by titration with nitrate of silver solution in 10 c.c., and expressed as chloride of sodium per cent.

Description of the Tyne and its Estuary.

The upper reaches of the North Tyne are fairly pure. The South Tyne is said to be polluted by a deposit of lead on the gravel, though the evidence of this is hardly conclusive. From the point where North and South Tyne join, the river runs for 18 miles and becomes tidal at Hedwin Stream between Carlisle and Ryton. The tidal portion of the river (see map) extends from this point for a distance of eighteen miles to the original bar, which is now dredged out, and beyond which two long breakwaters extend seaward for a distance of about a mile.



Into this tidal part Newcastle, Gateshead, Jarrow, South Shields, Tynemouth, and a large number of manufactories discharge their sewage and effluents, and four very much polluted tributaries enter. On the south side the Derwent from the Tyne enters between Scotswood and Benwell, the Team enters just above the Shot Factory in Newcastle, and the Don comes in above Jarrow Slake. On the north side the Ouse Burn enters at Newcastle.

The existence and position of these streams are of importance in understanding the tidal movements of the polluted water.

Particulars of the pollution discharged into the main stream will be found in an appendix to this paper.

As to the condition of the blind part of the river called Bell's Close

and of the two tributaries, the Team and Derwent, Inspector Dagg writes as follows:—

“The blind piece of river at Bell’s Close has only water in it when the tide is in the river: at low water it is dry. It was closed up as shown on the map by the Improvement Commissioners, who made a new watercourse.

“The tide goes some distance up both the Team and the Derwent. In the low part of the Team no fish are ever seen of any kind; it is very much polluted by sewage from several places, colliery water, and a brown-paper mill. The Derwent is the same. It runs through a thickly populated mining country, it has three paper mills, two white, and one brown-paper, and several other works on it. It is also very much polluted, and only at floods are fish seen in the lower part of it.”

The Dissolved Oxygen of the Water of the Estuary.

My observations on the amount of dissolved oxygen in the water in this estuary were carried out during the summer of 1901. The season was exceptionally dry, and as the summer advanced the volume of water in the river steadily decreased.

Owing to the pressure of other work it was found impossible to make more than a limited number of examinations or to extend the analyses beyond the determination of the free oxygen.

Method of Procedure.

On May 31st a preliminary survey of the river was made by my assistant Mr Patterson along with Inspector Dagg. Samples of water were then taken from certain places near the banks which the Inspector considered specially foul.

In all subsequent examinations the samples were taken from mid-stream and at a depth of three feet. These samples were taken at various points from the top of the tideway down to the original bar. These points are indicated on the map. As it was desirable to study the state of the river in different conditions of the tide, observations were made both at high and low water, sometimes on the same day, sometimes on different days.

Record of Observations.

I. The first examination was made on May 31st when water was collected at the following points about high tide.

1. Dunston Staithes, close to south bank.
2. Between High Level and Redhaugh Bridges at sewer outflow on north bank.
3. About 50 yards up mouth of Ouse Burn.
4. Bill Quay close to south bank.
5. Mouth of Don River at Jarrow Slake taken at commencing flood tide.
6. Mid-stream opposite Tyne Dock entrance taken at commencing flood tide.

These samples were not shaken with air but were examined on June 1st with the following results:—

	NaCl ‰	Free oxygen c.c. per litre	
		Individual observations	Average
1.	1·5	2—2—3	2·3
2.	2·0	2—2—2	2
3.	1·3	0—0	0
4.	2·5	3—4	3·5
5.	1·4	0—0	0
6.	2·5	4—4—5	4·3

II. The second examination was made on water collected on June 20th at low tide (overlapping spring), *i.e.* nearer spring than neap.

	NaCl ‰	Free oxygen c.c. per litre
Hedwin Stream	·02	6
Low Benwell Ferry	1·20	4
Shot Factory	2·16	5
St Peter's	2·12	5
Bill Quay	2·16	3
Jarrow Slake	2·64	5

III. A third examination was made on water collected on July 8th at high tide.

	NaCl ‰	Free oxygen c.c. per litre
Lower Benwell	1·88	3
Shot Factory	2·16	2·5
St Peter's	2·66	3·5
Bill Quay	2·60	4
Jarrow Slake	2·92	5

Pollutions of Tyne Estuary

IV. and V. The fourth and fifth examinations were made on water collected on July 16th, first at high and second at low tide (spring tide and lower ebb than 20th June).

	High Tide.			Low Tide.		
	NaCl ‰	Free oxygen c.c. per litre		NaCl ‰	Free oxygen c.c. per litre	
		Separate observations	Average		Separate observations	Average
Shot Factory	2.14	3—3	3	1.86	4—5	4.5
St Peter's	2.54	4—4	4	1.98	2—3	2.5
Bill Quay	2.64	4—6	5	2.16	3	3
Jarrow Slake	3.0	4—6	5	2.62	5—5	5
Original Bar	3.0	4—6	5	2.82	5—5	5

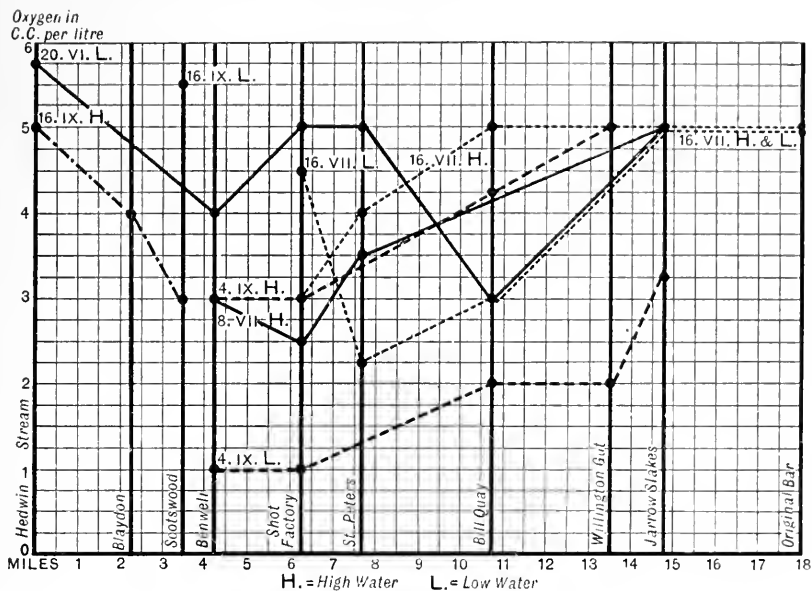
VI. and VII. The sixth and seventh examinations were made on water collected on September 4th at high and at low tide.

	High Tide.		Low Tide.	
	NaCl ‰	Free oxygen c.c. per litre	NaCl ‰	Free oxygen c.c. per litre
South Benwell	1.60	3	1.20	1
Shot Factory	1.74	3	1.40	1
Bill Quay	2.20	4	1.86	2
Willington Gut	2.66	5	2.26	2
Jarrow Slake	2.84	5	2.56	3

VIII. and IX. The eighth and ninth examinations were made on September 16th and 17th at high and at low tide.

	High Tide.		Low Tide.	
	NaCl ‰	Free oxygen c.c. per litre	NaCl ‰	Free oxygen c.c. per litre
Hedwin Stream	0	5		
Blaydon	1.68	4	Lost	Lost
Scotswood	1.80	3	0.76	5—5

From these tables the following diagram of the distribution of free oxygen in the Tyne Estuary at different parts of the summer has been constructed.



CONCLUSIONS.

The present observations are all too few, but they are published in the hope that they may induce others to take up the study of estuarial pollution. Although so limited they seem however to indicate pretty clearly the main difference between the Tyne and such a river as the Thames, and to explain how it is that salmon can pass through the estuary of the former.

The difference appears to be due to the fact that tributary streams enter the Tyne, and that the polluted water is forced into these by the advancing tide to be again poured out into the main stream as the tide recedes. A fairly pure waterway is thus provided at high tide, while at low tide if the amount of upland water in the main stream is sufficient, as in the earlier part of the summer, the impurities from the tributaries are diluted and partly washed out; but if the upland water is insufficient, as in September, these impurities make themselves manifest and cause a marked and serious diminution in the oxygen in the water of the main river.

While in the Thames the zone of pollution simply moves up and down with the tide and becomes more concentrated as it is forced back

at high water, in the Tyne we have found little evidence of such a concentration, and the river appears to become filled with the purer inflowing sea-water.

At high water the zone of least oxygenated water is between Benwell and Shot Factory, but even here the amount of oxygen does not fall much, if at all, below 50 % of the normal.

At low water the zone of greatest deoxygenation varies. On June 21st it was at Bill Quay, on July 16th at St Peter's, and on September 3rd from Benwell to Shot Factory. As the upland water decreased this zone of pollution extended upwards. In June the dissolved oxygen did not fall below 50 %. In July it fell only slightly below this, but in September it fell as low or lower than 16 %.

In conclusion I desire to acknowledge the very valuable assistance given me by Inspector Dagg, who not only collected all the samples of water analysed but also supplied very valuable information as to the state of the river and its tributaries. Without his intelligent co-operation these investigations would have been impossible.

APPENDIX.

EFFLUENTS (ALL UNTREATED) DISCHARGED INTO THE TIDAL PORTION
OF THE TYNE.

Site of Outfall	Nature of Effluent—i.e. whether Mineral, Chemical, Sewage or other Effluent
<i>A. On South Bank of Tyne</i>	
South Shields, between the Law and Tyne dock	Sewage from South Shields and Westoe, and effluent from Colliery and from Gas Works
The "Don" which enters the River and Jarrow Slake	Water from Boldon Collieries, effluent from Paper Mills, sewage from Tyne Dock, and East and West Harton villages, drainage from chemical refuse heaps
Jarrow	The whole of the sewage refuse from Gas Works, effluent from Chemical Alkali Works, Lead Works, Creosote Works, and drainage from chemical refuse heaps
Hebburn	The whole of the sewage effluent from Sulphur and Copper Works, Acid and Chemical Works, Lead and Paint Works, and Hebburn Colliery
Bill Quay	The whole of the sewage refuse from Lead and Paint Works, effluent from Colliery
Felling and along right bank of Tyne to Gateshead	The whole of the sewage effluent from Alkali Works, Brown-Paper Mill, and a large amount of drainage from chemical refuse heaps
The "Teams" entering at Dunstan	Effluent from Colliery, sewage from Dunston, Gateshead and Low Fell Teams and other villages. Effluent from Brown-Paper Mills, Creosote Works
The "Derwent" entering at "Derwenthaugh"	Effluent from various Paper Mills and numerous Collieries. Sewage from Swalwell and other villages
Blaydon	The whole of the sewage from Blaydon Stella and Addeson village. Effluent from Alkali Works, Manure Works, and Collieries
<i>B. On North Bank of Tyne</i>	
Tynemouth at the Black Middens	Part of the sewage from Tynemouth
North Shields	The whole of the sewage from North Shields, Flatworth, Percy Main. Effluent from Gas Works, Collieries, Lead Works, Creosote Works
Howden	The whole of the sewage and effluent from Lead and Antimony Works
Willington Quay	The whole of the sewage and a large filthy and acid discharge from Copper Works
Low Walker	The whole of the sewage effluent from Copper and Acid and Gelatine Works
Wallsend	The whole of the sewage effluent from Collieries and drainage from chemical refuse heaps
Bill Point	Effluent from Colliery and drainage from chemical refuse
St Anthony's	The whole of the sewage effluent from Lead Works and drainage from chemical refuse heaps
St Peter's	The whole of the sewage
Ouseburn	Sewage from several villages—Byker, Jesmond, and part of Newcastle
	Effluent from Collieries, Lead and Colour Works, Tanneries
Newcastle from Ouseburn to Elswick	The whole of the sewage effluent from several Collieries, from Tanneries, Lead Works, Gas Works
Delavel	Sewage from Delavel, Benwell, and South Benwell villages, and effluent from Colliery
Scotswood	Sewage from Scotswood and Denton Burn villages, and effluent from Colliery and Brown-Paper Mill
Lemington	Sewage from Lemington and Bells Close villages and effluent from Colliery
Newburn	Sewage from Newburn, Walbottle, Throckley, and Heddon villages, and effluent from three Collieries

ON AN OUTBREAK OF SORE THROATS AND OF SCARLET FEVER CAUSED BY INFECTED MILK.

By ARTHUR NEWSHOLME, M.D., F.R.C.P.

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THE outbreak about to be described raises issues of practical importance which appear to make it worthy of record, in spite of the fact that owing to fear of injury to a particular dairyman it was impracticable to complete the evidence by making inquiries at every house supplied with milk by him.

It will be convenient to describe the four branches of the outbreak in the order in which they came to my knowledge, subsequently placing these branches in their relationship to each other and to the milk, which could only be suspected when definite notifications of scarlet fever (group C, p. 155) gave the data required for pursuing an investigation.

Group A. About the 25th November I was consulted by Dr N., the medical attendant on the scholars at Miss S.'s day school. This school has 16 day pupils varying from 7 to 16 years of age. Two days earlier a boy M. S. aged 7 attending this school had been notified to be suffering from scarlet fever, date of onset Nov. 16th. For several days the doctor had been doubtful of the diagnosis, the symptoms being very mild. Dr N. now informed me that Miss S. was anxious to know what school precautions she should take; but at the same time expressed the opinion that M. S. had acquired his attack at a hippodrome performance in the town. She very wisely however gave Dr N. a list of sore throats in the school during that term. This list is embodied in the following tabular statement:

	Date of onset	Symptoms	Remarks
(1) Y. N. boy, aged 8	Oct. 29	sore throat	A slight attack.
(2) D. T. boy, aged 7	Nov. 6	sore throat	Discontinued attending school from the 6th to 13th Nov.
(3) N. Sp. girl, aged 11	Nov. 13	sore throat	Returned to school Nov. 17.
(4) M. S. boy, aged 7	Nov. 16	scarlet fever	
(5) S. P. boy, aged 9	Nov. 18	sore throat	Had vomiting as well as sore throat. Returned to school in a few days. Had scarlet fever a year earlier.
(6) K. Sp. girl, aged 13 (sister of N. Sp.)	Nov. 18	sore throat	

Further particulars were subsequently obtained about N. Sp. She was ill enough at the outset to be kept in bed for three days, but returned to school on the next day. Her father, who is a doctor, states that she had no rash. Some time after her return to school she was noticed to be picking rough skin on her fingers and was sent home. Her father was still of opinion that there was no true desquamation. Her sister K. Sp. failed with a sore throat on Nov. 18th, and the mother Mrs Sp. is stated to have had a severe sore throat apparently beginning on the same date as N. Sp. There are five children (varying in age from 14 to 5 years) and two servants in this house. Only one child has had scarlet fever previously, namely a boy aged 9, several years ago. He, a brother aged 5, a brother aged 14, the father and the two servants have not had sore throats recently. It is quite certain that with the possible exception of N. Sp. and the definite case of scarlet fever M. S., none of the above patients desquamated to any appreciable extent after their attacks of sore throat. The bearings of these cases on milk supply will be subsequently discussed.

Group B. On the 7th Nov. I received from Dr S. three swabs from sore throats. The result of the examination of growths on blood-serum was telephoned to Dr S. next morning, a pure culture of *Streptococci* having been found in each instance. I heard nothing more of these cases until Dec. 17th, when a letter from Dr S., of which the following are the pertinent paragraphs, was received :

I send a report of the epidemic of "streptococcus throats" which occurred at Miss C.'s school in November, the first three cases of which I took swabs of, and on which you kindly reported as shewing pure cultures of a *Streptococcus*.

In the course of the succeeding five days after the date of your report the number of cases rapidly increased and reached a total of 14. In three cases the symptoms of general septicaemia were severe, high temperature, rapid pulse, and greyish fibrinous exudate on the tonsils—none on the soft palate.

In two cases there was an evanescent rash very like the slight erythema seen

after an enema or a poultice. It lasted only a few hours, and there has not in any case been the slightest sign of desquamation.

The cases were all convalescent except one by Dec. 1st, and this one recovered completely by Dec. 4th, and there have been no subsequent cases.

On receiving Dr S.'s valuable letter, I arranged an interview with Miss C. and she supplied me with very full information as to the series of sore throats. For reasons which will be shortly apparent, it will be convenient to begin with case II., as case I. was only mentioned towards the end of the inquiry, and was only seen by Dr S. at a much later date.

II. On Nov. 4th Dorothy B. aged 10, had shivering at 4 p.m., was kept in bed next day, no sore throat until the 6th. No rash, except a slight rash on one day in the following week. She resumed her lessons at the end of three weeks.

III. Mrs B. mother of the above patient, had been out of the town from the 1st to the 4th Nov. On the 5th she was well until the evening, when she felt ill. In the night her temperature was 102° F. Next morning severe sore throat, high temperature. No subsequent desquamation.

IV. Miss W. aged 25 years, onset Nov. 6th with headache and sore throat.

V. Miss M. aged 16 years, onset Nov. 6th with headache and sore throat.

These two cases were much milder than II. and III. They remained in bed three weeks. No desquamation.

VI. On 8th Nov. Miss Mr. aged 11 years, failed with severe headache, sore throat and high temperature. Temperature remained elevated for several days.

VII. On 9th Nov. 4 cases began, viz. Miss Br. aged 12,

VIII. Miss A. aged 15,

IX. Miss M. Br. aged 8, and

X. Miss Bu. aged 10. These had similar symptoms to the other cases. Miss M. Br. had "acute tonsillitis."

XI. On the 10th Nov. Miss W. aged 9 began in a similar way.

XII. On the 11th Miss A. W. aged 11 began in a similar way.

On the 12th five cases began, some of them very slight, viz. XIII. Miss Mo. Br. aged 10. XIV. Miss J. B. aged 4½. XV. Miss Bl. aged 10, and XVI. Miss — Bl. aged 9.

Case XIII. had a slight erythema, but with this exception and case II. there was no rash. No desquamation occurred in any case. The

I. Miss J. æt. 11
onset Oct. 29

<p>II. Dorothy B. æt. 10 shivering Nov. 4 sore throat Nov. 6</p>	<p>III. Mrs B. onset late on Nov. 4</p>	<p>IV. Miss W. æt. 25 onset Nov. 6</p>	<p>V. Miss M. æt. 16 onset Nov. 6</p>
<p>VI. Miss M. æt. 11 onset Nov. 8</p>	<p>VII. Miss Br. æt. 11 onset Nov. 9</p>	<p>VIII. Miss A. æt. 15 onset Nov. 9</p>	<p>IX. Miss M. Br. æt. 8 onset Nov. 9</p>
<p>XI. Miss W. æt. 9 onset Nov. 10</p>	<p>XII. Miss A. W. æt. 11 onset Nov. 11</p>	<p>XIII. Miss M. B. æt. 10 onset Nov. 12</p>	<p>XIV. Miss J. B. æt. 4½ onset Nov. 12</p>
<p>XV. Miss B. æt. 10 onset Nov. 12</p>	<p>XVI. Miss B. æt. 9 onset Nov. 12</p>	<p>XVII. Miss M. Br. onset Nov. 21</p>	<p>XVIII. Miss M. Br. onset Nov. 21</p>

sore throats varied in severity from a slight sore throat to acute tonsillitis.

There were four servants in the house. The cook and one housemaid remained well, the parlourmaid and one housemaid had a "congested throat" during the week in which the majority of the above cases occurred. These patients' temperatures remained normal. Three governesses in the house and the lady principal remained well.

Owing to the preceding outbreak the school was temporarily broken up, resuming on the 21st Nov. On the evening of that day case XIII. who had come downstairs shivered, and was found to have a temperature of 103° F. followed by acute tonsillitis. Her previous attack had been slight, the temperature not reaching 100°; and she had only been kept in bed one day.

It will be noted that in none of the preceding cases did the symptoms lead to the slightest suspicion of scarlet fever. Diphtheria was suspected, but negatived both bacteriologically and clinically.

Further inquiry shewed that an earlier case had occurred—case I. Miss J. aged 11 goes home to another house in the town each Saturday to Monday. She returned to school on Monday, 28th Oct., looking poorly, and next morning vomited on the first-floor landing, after hurriedly leaving the breakfast table. She was laid on a bed in the bedroom occupied by cases II. and III., and she again vomited while lying on III.'s bed. Her dress was changed while lying on this bed. She remained on it until the evening, when she returned to her own bedroom (3rd floor east): her temperature on this day was 100° F., next day 99°. Next day she returned to the bedroom of cases I. and II. which was used as a sitting-room by convalescent patients. Miss J. was not very ill, but is stated to have had slight sore throat and a little fever. No rash was noticed, and no desquamation occurred. She was specially examined by the doctor for desquamation. As Miss J. was subject to "bilious attacks" little would have been thought of this attack had it not been followed by unusual weakness and a subnormal temperature. The probable relationship of the series of cases to each other is brought out more clearly by the scheme on p. 153.

The most likely explanation of the outbreak appeared to be that case I. brought the infection—the nature of which will be more conveniently discussed later—into the school, that she passed it on to cases II. and III. and probably to other girls who frequently entered the same room. The bedroom accommodation of patients and of pupils who remained well was as follows:

Bedroom on 1st floor	{	Case I.	One girl sleeping in this room remained well.
		Case II.	
		Case III.	
2nd floor west	{	Case VI.	Two girls sleeping in this room remained well.
		Case XII.	
2nd floor east	{	Case V.	Two girls sleeping in this room remained well.
		Case VIII.	
3rd floor west	{	Case X.	
		Case XV.	
		Case XVI.	
3rd floor east	{	Case IV.	
		Case VII.	
		Case IX.	
		Case XIII.	
		Case XIV. and Case I.	
3rd floor east, 2nd room		Case XI.	

Thus the total number of boarders was 19, of whom 14 were ill.

The	"	"	teachers	"	6	"	"	2	"	"
"	"	"	servants	"	4	"	"	2	were slightly	ill.

In addition there were six day boarders, all of whom have remained well. These arrive at 9.30 a.m. and leave at 5 p.m. They all attended school until the 6th of November, subsequently being kept at home until the 21st Nov. They take the same food including milk as the boarders. They do not however go into any bedroom except once a week to change clothes for a dancing class. If the infection be assumed to be located in the bedrooms, it was likely therefore that the day scholars would escape, as at the most they only entered a bedroom once after the cases began; and this was not a bedroom in which a sore throat had already occurred.

Group C, consisted of cases of definite scarlet fever. The relationship between the total amount of scarlet fever in the town and in group C. is brought out by the following weekly statement for the last two months of the year, in which dates of onset are taken instead of the somewhat irregular dates of notification.

Week ending	Total cases	Group C	Week ending	Total cases	Group C
Nov. 2	6	—	Dec. 7	6	5
" 9	3	—	" 14	2	—
" 16	2	—	" 21	3	—
" 23	2	2	" 28	4	—
" 30	3	—			

As already related one case of scarlet fever (M. S. a boy aged 7) occurred in connection with group A. For convenience this case is restated in the following list:

			Date of		Disease
			Onset	Notification	
Group C	1st Section	{ M. S. boy, aged 7	Nov. 16	Nov. 25	Scarlet fever ¹
		{ W. C. „ „ 12	„ 18	„ 20	„
	2nd Section ²	{ E. W. girl, aged 14	Dec. 2	Dec. 5	„
		{ E. H. B. „ „ 7	„ 3	„ 6	„
		{ E. S. woman, „ 45	„ 4	„ 9	„
		{ A. P. „ „ 40	„ 4	„ 9	„
		{ H. B. man „ 24	„ 5	„ 9	„

At the time when the two first of the above cases were notified no definite history of infection could be ascertained. The two patients did not know each other, lived in different streets, and attended different schools. At M. S.'s house there was a sister aged 4, father and mother, and several servants, among whom no sore throat or other symptoms of illness occurred. At W. C.'s house there is a mother, a governess, two sisters aged 10 and 15 years, and two servants, none of whom had sore throats at or near the date of onset of W. C.'s attack of scarlet fever. As the home supply of milk in these two houses was different, no suspicion as to milk infection was entertained.

On the 5th Dec. I was asked by the doctor in attendance to see E. W., a girl aged 14, who was then suffering from an intensely severe attack of scarlet fever with an unusually bad type of sore throat. The other persons then living in this house were father and mother and three servants, who none of them had sore throats or other symptoms of illness about this time. School infection and other possibilities of personal infection were apparently excluded.

E. H. B. aged 7, the daughter of a doctor, was poorly on the evening of Dec. 3rd, vomited in the night; during the next two days she was better, but on the 5th complained of sore throat, and on the 6th a scarlatinal rash appeared. Otorrhoea followed in a few days. No evidence of personal infection. At a later date the following further facts were ascertained. The father on the evening of the 4th Dec. was suddenly seized with giddiness and cold sweat. Next morning he had

¹ Also given as (4) Group A.

² In the neighbouring town of H. to which the dairyman P. (see p. 158) also supplies a portion of his milk one case of scarlet fever in which his milk was drunk was notified on Dec. 6th.

a slight sore throat, which was much worse on the 6th and 7th. He continued at his work; his temperature was not taken. There was no rash and no subsequent desquamation. He has noticed that he has suffered from similar sore throats on former occasions when attending scarlet fever patients. He has never had scarlet fever. The child of the last patient, a boy aged 10 months, vomited on the evening of the 3rd Dec., and had diarrhoea and was "sadly" for five or six days. His throat was examined, but nothing abnormal was discovered. There was no dysphagia and there were no enlarged glands, no rash, and no subsequent desquamation. A boy aged 4 who has not previously had scarlet fever remained well. The mother, who has had scarlet fever, also remained well. There are four servants in the house all of whom have remained well.

E. S. aged 45, failed with scarlet fever on the 4th Dec. No history of source of infection could be obtained. No children live in this house, but six other female adults, concerning whom it has since been ascertained that they had no sore throats or other symptoms of illness near the date of onset of E. S.'s attack. Her attack was a dangerous one.

A. P. aged about 40, failed with scarlet fever on Dec. 4th. She is a lady district visitor, but no cases of scarlet fever have recently been notified in the district in which she visits. This also was a very severe case. The only other persons living in this house were an adult female cousin and two servants, all of whom have remained well.

H. B. aged 24, failed with scarlet fever on Dec. 5th. He was in London from the 30th Nov. to 2nd Dec., but was not known to have come in contact with a case of infectious disease. There were living in the same house the patient's father and mother, sisters aged 12, 19 and 20 years and a brother aged 16 years, and two servants. The mother and two servants had slight sore throat about the time of onset of H. B.'s attack of scarlet fever. None of them were sufficiently ill to ask the doctor to see them.

The preceding patients all lived in houses and streets remote from each other. No other cases of scarlet fever were known to exist at the time in the neighbourhood, except the two given in the table (group C. sec. 1), and these were carefully isolated. In only one of these two cases was the home supply of milk from P. On Dec. 9th however when the three last cases in group C. sec. 2 were notified, strong suspicion was aroused that the milk supply from P., which was common to all the seven cases in group C. except, as was then supposed, the first, might be at fault. A visit was therefore made to the farm from

which the majority of the dairyman P.'s milk is supplied, and on the strength of the information obtained at this visit immediate arrangements were made for keeping two milkmen away from their work.

The result of a more detailed inspection of P.'s dairy and employ  s which I made on the following day is appended :

Account of visit to P.'s farm, December 10th.

Mr P. states that he distributes from 330 to 350 gallons of milk a day in the town from his own farm at Z, and about 64 gallons a day from other sources. Thus between November 21st and December 9th he has had milk from seven farms, the milk coming almost daily from two of these farms. One of these auxiliary sources of supply is a creamery which collects milk from a large number of farms.

Seven families live in cottages close to Mr P.'s cowsheds. Among four of these families, including five children, there was no history of recent illness. The members of the other three families were examined with the following results.

T. K. and his wife, a girl aged 7, a girl aged 10, and a boy aged 16 form one of these families. On October 30th Edith K., the girl aged 10, had an attack which began with a headache. There is stated to have been no rash. She was examined by a doctor who said she had influenza¹. On returning to school at the end of a fortnight she was examined by the Medical Officer to the School Board, for evidence of possible diphtheria. He did not detect anything wrong. No cases of scarlet fever have since occurred at this school. K. milks once a day. The other children at this house have remained well. Edith K. had scarlet fever when a year and eight months old. The children are stated never to take cold milk. The milk is generally boiled and they drink it chiefly in tea.

Next door live L. and his wife and two girls aged 9 and 5. Dorothy L. aged 5, began with a sore throat and enlarged cervical glands on or about November 2nd, three days after the girl Edith K. The mother when further questioned stated that E. K. began with a

¹ This doctor subsequently sent me the following letter :—"I saw K.'s child on 1st Nov. suffering if I remember right from a mild influenza. There was certainly no throat affection or anything else suspicious. I gave directions for treatment and told them to let me know if the child did not at once get better, and I heard no more. The reason K. sent for me was, I think, that she was afraid it might be something infectious, as P. is very particular."

"cold in her head," and that she often has enlarged glands in the neck. She also states that not much milk is drunk and chiefly in tea. Mrs K. informed me that there was definite sore throat when Dorothy L. was ill.

Mrs B. who lives next door to Mrs L. on one side (Mrs K. living next door on the opposite side) informed me that when she first came to this house the L. children frequently came in to play with her baby, and Mrs L. said to her, "I know your baby will have it, as they have all had it up here." Mrs L. also showed Mrs B. some peeling of her child's hand, and when Mrs B. saw this she said, "I should certainly have advice." Mrs L.'s answer was, "I do not want any advice if the child is not downright ill."

Mrs B. noticed at this time there was thick peeling on the palm of one hand and new skin coming up underneath. She also said that there was a similar state of things on the back of the same hand. It is stated however by the mother that this desquamation followed a sore place on the hand.

B. is a young man aged about 25, living in a cottage with his wife, and a baby 6 months old. They came to this house from a house in the neighbouring town, on November 2nd. The baby began to be ill on November 4th. She had a bad cough. There is said to have been no rash or sore throat. Mrs B. began with a bad cold about a week later. She had a cough and slight sore throat and was husky. B. himself had a sore throat beginning on November 30th, which hurt him in swallowing. Examined on Decr. 10th he had a suspicious looking tongue and an injected throat. He occasionally drinks cold milk. His baby is breast-fed and Mrs B. does not drink milk.

After my visit and the exclusion of the B. family, the L. family and the K. family from any communication with the dairy, no further cases of scarlet fever definitely connected with this milk supply occurred.

Statement of Evidence connecting P.'s milk with the cases in groups A. B. and C.

It must be admitted that the illnesses among the three families living in cottages adjoining the farm dairy were slight and atypical. In none of them could it be asserted with a high degree of probability that scarlet fever had occurred, unless regard be had to the circumstances in connection with groups A. B. and C. to be now explained.

The cases of scarlet fever in group C. did not alone justify a

dogmatic statement that the infection was acquired from P.'s milk. Two cases Nov. 16—18 and five cases Dec. 2nd—5th in a milk supply averaging 330—350 gallons daily (of which the greater part was distributed in the town, and only a small portion in the neighbouring town H., in which one case of scarlet fever was notified on Dec. 6th) were fewer than might reasonably be anticipated in accordance with past experience of milk scarlatinal epidemics. Furthermore the last case directly ascribable to milk failed on Dec. 5th, while my measures of exclusion of the cowmen belonging to suspected or infected families did not take effect until the evening of Dec. 9th. Assuming the milk to be infectious, it was clearly only so spasmodically and at irregular intervals.

Assuming however that P.'s milk had caused the seven cases of scarlet fever occurring among his customers (group C. sec. 1 and 2) it became a matter of importance to determine whether the 270 to 290 gallons from his own farm or the 64 gallons coming from seven other farms had conveyed the infection. Three lines of inquiry were open. (*a*). The method of distribution of the milk from the different sources might possibly have helped. No records had however been kept of the method of distribution of milk from different sources. The milk from other farms than his own had been used by P. according to daily varying requirements, his own milk or his milk mixed with milk from these other sources being distributed in a manner which varied from day to day. (*b*). The necessity for an investigation at each farm was avoided by the discovery of (*c*) a crucial case. This was the very severe attack of E. W. (group C. sec. 2). Some months previously her father had complained of his milk supply, and it had always subsequently been sent direct from P.'s farm to his house in a padlocked can. As the simultaneous occurrence of scarlatinal infection in more than one farm supplying a dairyman was highly improbable, and as E. W.'s attack if caused by infected milk was caused by the milk from P.'s own farm, it was henceforth assumed that we had only P.'s milk to deal with.

We may now proceed to strengthen our chain of evidence by referring to groups A. and B. It will be convenient to refer first to group B. As will be remembered my attention was first drawn to the outbreak of sore throats at Miss C.'s school by a letter reaching me on Dec. 17th.

On interviewing the head teacher of this school I was informed that the milk supply was from X. dairy, a totally different source from P. This fact appeared at first to negative any connection between

groups B. and C. But the first patient in group B. was Miss J. (p. 153). This patient went home from Saturday to Monday and failed early on Tuesday morning. The milk at her home was supplied by P. and she had drunk this milk at home. No further inquiries could be made, but apparently Miss J.'s father and mother, infant brother, and the servants at her home had remained well. Assuming that she was infected by P.'s milk, the series of cases in group B. is comprehensible on the supposition that there was a direct transference of scarlatinal or some other form of infectious sore throat from her to them. No other source of infectious sore throat could be detected at this school; and I had no hesitation in linking the 16 cases in group B. on to the outbreak due to infected milk, through the intermediation of case I. (Miss J.), especially after I had re-investigated group A. in the light of the facts discovered as to groups B. and C.

Group A. consisted of 5 primary and 1 secondary case of sore throat (one of the five being undoubted scarlet fever) occurring among 16 day pupils. Seven of the pupils at this day school take unboiled milk at 11 a.m. This milk is supplied by P.

Of the seven who drink milk at school

- 2 had sore throat (2) (5),
- 1 had scarlet fever (4)
- 4 remained well.

Of the nine who did not drink milk at school

- 1 had a slight sore throat (1)
- 2 had a more severe sore throat (3) and (6),
- 6 remained well.

It appeared therefore improbable that P.'s milk had caused the outbreak. Further inquiry seemed to shew that case (1) was an ordinary sore throat (Oct. 19th) probably independent of the other cases. On questioning the doctor, who is the father of patients (3) and (6), it was ascertained that *P. supplies his household with milk*. These children therefore partook of the presumably infected milk at home. This important fact increases the probability that the cases in group A. were due to the same infection as groups B. and C.: and I am I think justified in view of the cumulative evidence which has been adduced, in inferring that this was probably the fact.

The three groups of cases may now be examined on the assumption that they were caused by P.'s milk, and their facts investigated from this standpoint. Their relationship in point of time to the cases on P.'s farm is shown in the following scheme.

(1). *As to Dates of Onset of Cases.*

Cases on P.'s farm	Group A	Group B	Group C	Cases on P.'s farm	Group A	Group B	Group C
	(Oct. 29)	Oct. 29			Nov. 13...		
Oct. 30		Secondarily infected cases			Nov. 18		
Nov. 2		Nov. 4			Nov. 16 (scarlet fever)		
(?) „ 4		„ 5			Nov. 18	Nov. 18 (scarlet fever)	
	Nov. 6	„ 6		Nov. 30			
		„ 6					
		„ 8					
		„ 9				Dec. 2 (do.)	
		„ 9				„ 3 „	
		„ 9				„ 4 „	
		„ 9				„ 4 „	
		„ 9				„ 5 „	
		„ 10				„ 6 „	
(?) Nov. 11		„ 11				„ 6 „	
		„ 12				(in another sanitary district)	
		„ 12					
		„ 12					
		„ 12					

The first case in group A. I regard as probably not belonging to the outbreak. The first case in group B. undoubtedly did belong to it: and it will be noticed that she failed a day prior to the first known case on P.'s farm, which *ex hypothesi* infected the milk. On the hypothesis of milk infection, either the dates must be wrong, which I think can be excluded, or an earlier case of infectious illness on the farm was not discovered, or the outbreak was caused by bovine disease independent of human infection. When the facts were investigated early in Dec. no evidence of udder disease was found, and fairly frequent veterinary inspections of the dairy had been made. I am inclined to think there was an earlier undetected human case of infectious sore throat. It was found that the children of the farm labourers had occasionally run in and out of the dairy, and been close to the cooling apparatus, and opportunities for infecting milk probably had arisen.

(2). *As to Multiplicity of Infection of Milk.*

If the milk caused the cases given in the preceding scheme, these cases including those on the farm divide themselves into three groups:

I. Cases originating Oct. 29th to Nov. 6th. In these cases symptoms resembling influenza occurred, or there was more or less

severe sore throat like the *Streptococcus* group B. (It will be remembered that all the cases in group B. are regarded as secondary to the case on Oct. 29th.)

II. Cases originating Nov. 12th to Nov. 18th. Two of these were scarlet fever: one was suspected of desquamating, and one other had only sore throat. This case however (group A. (5)) had had scarlet fever a year previously. He vomited at the onset of the present attack: and altogether his attack may be regarded as conforming to the more truly scarlatinal type shewn by the cases in group II. than those of group I.

III. Cases originating Nov. 30th to Dec. 6th. These with the possible exception of the farm labourer B. (onset Nov. 30th) were all true scarlet fever, most of them severe cases.

There was thus an increasing virulence of infection. B.'s condition when examined by myself on 10th Dec. I regarded as very suspicious. He could not remember whether he had suffered from scarlet fever in childhood.

It appears likely that there were three occasions on which the milk became infected. The total amount of infection must have been small or its infectivity slight, in view of the small proportion between the total number of cases and the volume of milk consumed. On the first occasion, only about 6 primary cases, including the farm cases, with 15 secondary cases are known to have arisen. On the second occasion four primary cases, two of them certainly scarlatinal, one other probably so, and one occurring in a boy partially protected by a previous attack of scarlet fever, and one secondary case occurred. This second group cannot be traced to any recent cases on the farm. The third group in my opinion was caused by infection from the farm labourer B. and all the cases belonging to it were severe scarlet fever.

(3). *As to the Amount of Infection.*

In calculating this, it will be advisable to omit all secondary cases, especially the 15 secondary cases in group B. The case in brackets at the head of group A. and the two farm cases marked ? are also omitted. With these deductions there were from Oct. 29th to Dec. 6th 16 cases in a milk supply averaging 330 to 950 gallons daily. Of these 7 were notified as scarlet fever, two others were very suspicious. The facts thus stated show that assuming the milk to have been the source of infection, the amount of infective material conveyed by it was small,

and the conveyance was only on exceptional occasions. These facts appear to me to exclude bovine infection, and to favour such casual human infection as may have arisen from P.'s workmen and their children.

(4). *As to the Character of the Infection.*

Were all the sore throats as well as the officially notified cases scarlatinal, or were two infections operating? The gradually increasing virulence of the cases, first sore throats, then sore throats mixed with undoubted scarlet fever, then a group composed entirely of cases of severe scarlet fever, supports the first view.

Some light may be thrown on this problem by the facts as to protection by a previous attack of scarlet fever of those attacked during this outbreak. Circumstances made it very difficult to obtain complete information under this head.

No information was obtained as to the children of P.'s labourers, except that the first case (supposed influenza) had scarlet fever eight years previously. In group A. one patient with sore throat had scarlet fever a year earlier. Cases (3) and (6) of this group, the former of whom was suspected of desquamating, had not had scarlet fever previously. In two cases of sore throat in this group the facts could not be ascertained.

In group B. complete data were similarly not obtainable. It is certain however that cases 2, 3, 7, 9, and 11 to 16 inclusive had not previously had scarlet fever, *i.e.* in 10 out of the total 16 cases in this group the fact that the attacks did not assume the typical type of scarlet fever was not due to a previous attack of scarlet fever. The cases in this group were undoubtedly infectious; from the throats of three of them pure cultures of *Streptococci* were obtained, which reminded me at the time of similar cultures from scarlatinal throats, and I incline to the view that the cases were truly scarlatinal although the only rash noticed was an evanescent erythema in two out of the 16 cases, and no desquamation was apparent in any case.

If this view be accepted, the most remarkable feature of this outbreak is the large proportion of cases of scarlatinal sore throat (*sine scarlatina*) which occurred.

Thus

	Scarlatinal Sore Throats.	Scarlet Fever.
Group A.	4 (or 5)	1.
Group B.	1 (primary case) 12 (secondary cases)	0.
Group C.	?	7 (or 8).

The facts as regards scarlet fever are complete. The same cannot be said as to cases of sore throat. There may have been a considerable number of such cases in this milk supply of which I have no knowledge. The outbreak illustrates the desirability of notifying all anomalous and untraced attacks of possibly infectious disease outside the present limit of the Infectious Disease (Notification) Act to the Medical Officer of Health. This would enable him in many instances to trace sources of infection much earlier than is now practicable.

If the same contagium caused the sore throats and the attacks of scarlet fever, it is evident that infected milk may carry the scarlatinal contagium in such an attenuated form or in such a minute amount that it is not capable of causing all the phenomena of scarlet fever. In group B. many of the attacks were most severe and septicaemic in type. They were apparently infectious: and yet not a single typical case of scarlet fever occurred.

Comparison with other Milk Outbreaks.

It will be useful in conclusion to contrast the experience in the above outbreak with certain well-known milk outbreaks of scarlet fever, as regards

(a) Proportion between families supplied with infected milk and the number invaded by scarlet fever.

(b) Duration of outbreaks.

(c) Occurrence of sore throat without clear evidence of scarlet fever.

The facts enabling this comparison to be made are embodied in the following table (p. 166):

(a). It will be noted that the lowest percentage of families supplied with the infecting milk who were invaded was 4 per cent. (Newcastle-on-Tyne), the highest 67 per cent. (Wimbledon). In the outbreak described in the preceding pages only 7 cases of scarlet fever were notified. If we add to these the 4 primary cases of sore throat in group A., the one primary case in group B., and all the 4 suspiciously invaded families connected with P.'s farm, the total number of cases is only 16. P. supplied 890 families, and the percentage of families implicated is 1.6. As already pointed out, no house-to-house investigation was made among P.'s customers, and probably more sore throats than those recorded above occurred. A similar state of things must almost certainly have occurred in many of the outbreaks with which comparison is made.

Date of outbreak	Locality	Reporter	(a)			(b) Duration of cause of infection	(c) Occurrence of sore throat apart from scarlet fever
			No. of families supplied by milkman	No. of such families invaded	Per- centage		
¹ June 1867	Penrith	Dr M. W. Taylor	14	6	42	A few days	None reported
¹ April—May 1877	New Barnet	Dr C. E. Saunders	135	58	43	128 cases between Apr. 29 and May 4	140 total cases and 12 of sore throat
¹ May—June 1879	Newcastle-on- Tyne	Dr H. E. Armstrong	350	14	4	Not clear from sum- mary	None reported
² Aug. 1879	Fallowfield near Manchester	Dr H. Alry	60 or 70	18	30 or 23	Of 35 total cases 24 within 36 hours	Two cases of sore throat reported
¹ Jan. 1881	Halifax	Dr Ainley	135	53	39	In 39 out of 51 houses in which the date of invasion was known, it was be- tween Dec. 29 and Jan. 8	Of 53 invaded households, 35 had scar- latinal cases and 18 had cases of sore throat; and among the 35 households in which definite scarlet fever was re- cognised, sore throat occurred in other members of the family in 9 instances. The epidemic is described as one of scar- latina and "throat illness." Mr Power remarks: "some (medical practitioners of the district) had noted now and again among attacks of comparatively speaking trifling sore throat occurring in fever- stricken households and elsewhere, anomalous cases, the precise nature of which might, but for this relation with the scarlatina, have been open to doubt"
³ Dec. 1880 to Jan. 1881	Wimbledon	Mr Cooper and Mr Power	273	174	67	152 families altogether were attacked, but of these 58 were supplied by counter- trade, and the number of families out of which these attacks occurred could not be ascertained	
⁴ Aug. 1892	Glasgow	Drs J. B. Russell and Chalmers	359	94	26	1st to 12th Aug.	
⁵ 1900	Clifton	Dr D. S. Davies	269	42	16		

¹ Extracted from "The Influence of Milk in Spreading Zymotic Disease," by Ernest Hart, *Trans. Internat. Med. Congress, London, 1881*, Vol. iv. p. 491.

² *Ann. Rep. of the Med. Off. of the Local Gov. Board for the year 1879*, p. 93.

³ *ibid.* 1886, p. 327.

⁴ *Glasgow Med. Journ.* Jan. 1893, p. 1.

⁵ *Journal of Hygiene*, Vol. i.

(b). In duration of the cause of infection, as judged by the dates of notification of cases, the present outbreak is very exceptional, and it appears to lend itself best to the supposition given on p. 162 of three successive infections of the milk.

(c). In many of the other outbreaks sore throat apart from other evidence of scarlet fever frequently occurred alongside of definite cases of scarlet fever. So far as I can ascertain, however, the present outbreak is unique in regard to group B., unless it be maintained that the members of this group were not secondarily infected by the milk-infected sore throat of Miss J. (group B. I.). This must remain to some extent a matter of opinion. In view of all the facts, I am of opinion that group B. consisted of scarlatinal sore throats occurring chiefly among girls unprotected by previous attacks of scarlet fever, and that Miss J. introduced this mitigated infection into the school.

The following two outbreaks may be adduced in conclusion. The late Dr (afterwards Sir R.) Buchanan describes¹ an outbreak in Kensington in which 12 persons "were attacked with scarlet fever and six others with sore throat or with sore throat and other symptoms resembling scarlatina" within 5 days of June 9th, 1875. Dr Buchanan adds, "I note that 4 persons who had not to their knowledge had scarlatina before and who were exposed to circumstances apparently identical with those that produced scarlatina in 13 others, had no scarlatina rash, but some form of sore throat: one of these four having serious laryngeal symptoms." This outbreak was traced to infected cream.

Dr J. K. Warry² reports on "a recent outbreak of septic sore throat disease apparently caused by infected milk." The outbreak as near as could be ascertained prevailed during nearly the whole of April and the first week in May. This protracted duration may be compared with the dates given on p. 162. In ten cases in this outbreak observed by one doctor, the symptoms were tonsillitis (not follicular), with considerable swelling of the cervical lymphatic glands, an elevated temperature lasting in nearly all the cases for at least a fortnight, great prostration, in one case acute septicaemia ending in septic pneumonia and death, in two cases acute nephritis, etc. In some of the families the suspected milk was always boiled before use. No complete notification of cases could be obtained, but in two areas a house-to-house visitation was made with the following result:

¹ *Report of Med. Off. to the Loc. Gov. Board*, New Series, No. VII. 1876, p. 72.

² *Annual Report, Borough of Hackney*, 1900, p. 60.

	No. of Houses.	Percentage Households affected with sore throat.	
		(a) Among households supplied with the suspected milk	(b) Among other households
Area A.	168	29.1	2.0
Area B.	75	14.2	0.0

In none of the implicated families did any recognisable cases of scarlet fever occur. I place the bare outline of the interesting outbreak described by Dr Warry on record, but am unprepared to give an opinion as to whether they were "septic sore throats," or were sore throats like those of group B. in the present outbreak, which I have preferred to regard as scarlatinal in nature. If my view as to group B. is correct, it opens up a vista of increased difficulty in the recognition and therefore in the prevention of scarlet fever. That this difficulty must be recognised and admitted, as we already recognise and admit it in the case of diphtheria, is I think an important practical inference from the facts narrated in the preceding pages.

Conclusions.

If the view taken as to the connection between the groups of cases described in this paper be correct, the following conclusions are suggested:

1. Scarlet fever may be caused by infected milk containing the contagium in such an attenuated form or minute quantity that no symptoms manifest themselves except an anomalous sore throat with fever.

2. Scarlet fever may assume this type in a large number of children who have not been partially protected by a previous attack of scarlet fever.

3. If such anomalous cases occur among milkmen or their families the milk may be infected at intervals for a much longer time than has been recognized in previously described milk-outbreaks of scarlet fever and scarlatinal sore throat.

4. The fact that only a few cases of scarlet fever are traceable to a given milk supply does not necessarily shew that this milk is not infective. The fewness of the cases in this outbreak, and their sporadic character, is analogous to the suspected connection between sporadic cases of enteric fever in the metropolis and the presence of excessive amount of organic matter in the metropolitan river water-supply

(Corfield) or the occurrence of floods a fortnight before the onset of the cases in question (Shirley Murphy). In each instance the dose of the contagium is small, and the detection of causative connection between the infecting material and the cases of disease is difficult. The demonstration of the connection is impossible.

5. The occurrence of anomalous attacks of sore throat, as in this outbreak, indicates the desirability of the notification of all such cases to the Medical Officer of Health. He would by this means be placed in a much more favourable position to trace sources of infection. My views on this subject are set out in full elsewhere¹.

¹ "A National System of Notification and Registration of Sickness," *Journ. Roy. Statist. Soc.* Vol. LIX. Part I.; and "Possible Medical Extensions of Public Health Work," *Journ. State Med.* Sept. 1901.

THE MEASURES TAKEN TO CHECK THE DIPHTHERIA OUTBREAK OF 1901 AT COLCHESTER.

(One Map and Four Charts.)

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[Thesis for the Degree of M.D., University of Cambridge.]

The History of the Epidemic.

THE epidemic of diphtheria in Colchester, which reached its height in the Summer of 1901, followed almost without intermission on the outbreak in the Autumn of 1900.

The disease during the Autumn of 1900 was however mostly confined to Old Heath, a suburb of Colchester, situated to the south-east of the town, and first made its appearance in August, when three persons notified to be suffering from diphtheria were treated in the Mile End Infectious Hospital. During the months of September, October, November, and December, 2, 11, 5, and 8 patients respectively were treated there.

This outbreak was followed by a period of four and a half months (January 1st to May 11th) in which the mean notification rate for diphtheria dropped to one per week.

It was towards the latter half of May that the epidemic, which is the subject of this paper, first began to assume serious proportions, thirteen cases being notified during the week ending May 25th, and twenty-six during the whole month. By this time, however, the disease was no longer confined to Old Heath, but had reached the town of Colchester. It rapidly increased during June (46 cases), when the highest number of notifications for one week, namely 22, was reached.

It is of special interest to note that though a few cases during May and June occurred in other quarters of the town, the great majority came from the south-eastern portion adjoining Old Heath. Through May and June 72 cases in all were notified, and of these 80 per cent

occurred in the south-eastern district, and 43, or 60 per cent., in a small area, bounded by Magdalen Street, Wimpole Lane, Canterbury Road, and Military Road, in the centre of this district. (See Map, p. 172.)

In July there was a still further increase, 66 cases, the maximum for any one month being recorded. During this and the following months the notifications were no longer restricted to the district just mentioned but were received from all sides.

The first decided fall in the notification rate occurred in August with 38 cases, and about the same mean level was maintained during September and October, with 35 and 32 notifications respectively.

A still further reduction took place in November, when 15 persons were notified as suffering from diphtheria, and again in December, when 8 only were recorded up to the 28th.

As indicated in the above short history of the outbreak, the epidemic, contrary to common experience, was a Summer one.

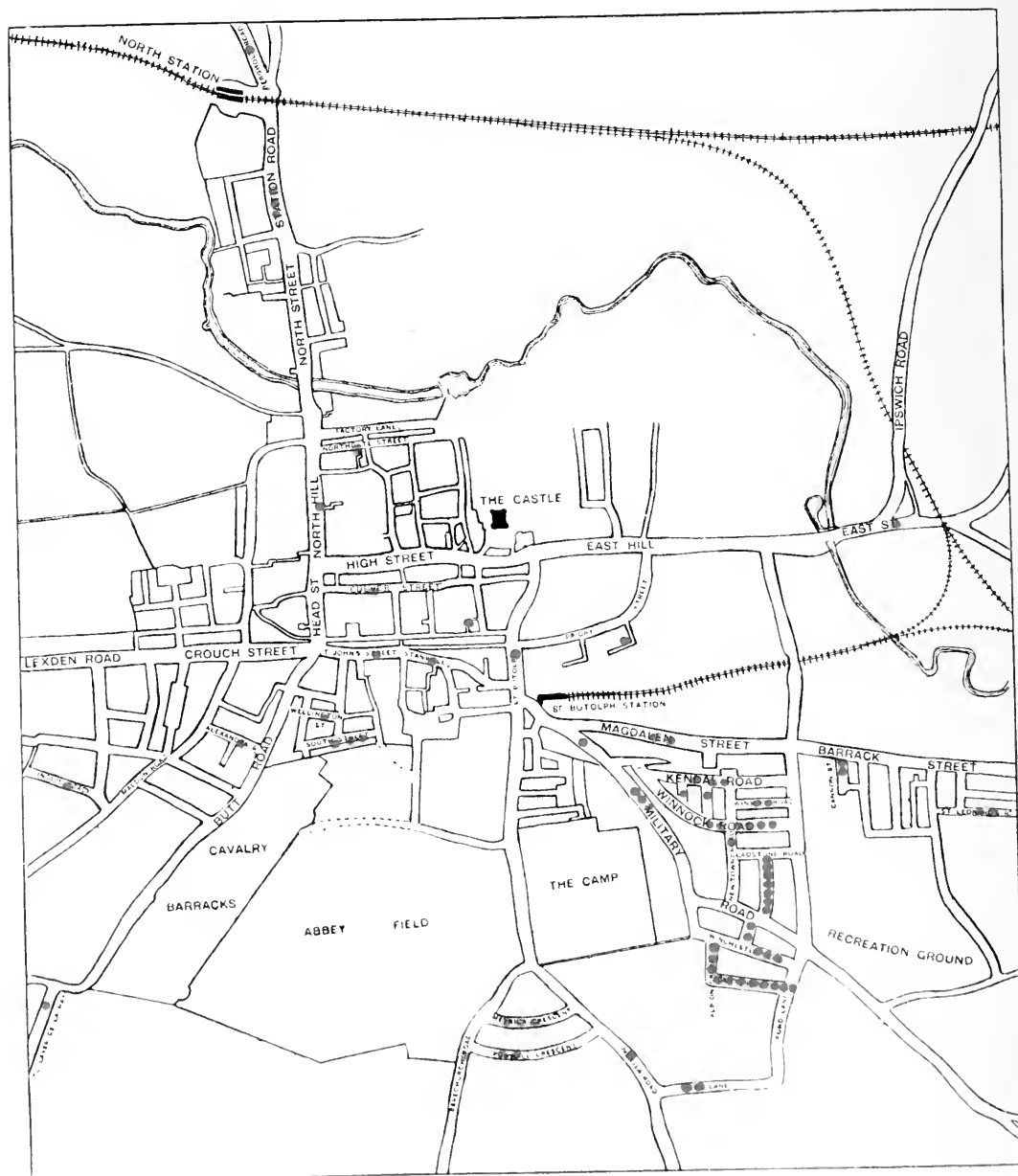
Not only was the disease widely spread, and the mean notification rate thirteen per week for the past two months, but the unhealthy period of the year was rapidly approaching before the measures suggested by Dr Cobbett for limiting the spread of the disease were employed. The efficacy of the measures was therefore put to a severe test, and the good results following their adoption are the more noteworthy.

In this connection I should like briefly to point out that the decline in the disease during August followed on the resolution of the Sanitary Committee to detain all the patients suffering from diphtheria in the Mile End Hospital until three successive negative bacteriological examinations had proved them to be free from diphtheria bacilli, and that the November fall followed on the more complete application of the precautionary measures.

Treatments employed in the Mile End Hospital.

In considering the treatments adopted the year should be divided into two halves.

Before July 16th antitoxin was not given as a routine treatment on the admission of the patient to the Mile End Hospital, but appears to have been administered on arrival to a few serious cases only, and to some others who developed alarming symptoms later. During this period reliance was placed on antiseptic sprays to destroy the bacilli in



MAP OF COLCHESTER.

the throat. By such methods 81 cases were treated, of whom 21 died. The case-mortality was therefore 25·9 per cent.

After July 16th antitoxin was administered in accordance with a resolution of the Sanitary Committee to every case as soon as possible after admission, unless this had been previously done, and no efforts were made to destroy the bacilli in the throat.

Between July 16th and December 28th, 119 patients¹ were treated in the Mile End Hospital on this plan, and seven died. For this period the case-mortality was accordingly 5·8 per cent.² (This does not include certain cases the notification of which as cases of diphtheria was subsequently withdrawn and that of scarlet fever substituted, but it includes cases in which no diphtheria bacilli were found, for it was possible to exclude these only from the second group, and it was desirable that the two periods should be strictly comparable.)

The fall in the case-mortality from 25·9 to 5·8 per cent., which occurred on the introduction of the systematic use of antitoxin, affords a striking example, if any were needed, of the value of that remedy.

This fall has been ascribed to a gradual diminution in the severity of the disease. The accompanying chart (No. I.) of weekly admissions and deaths from diphtheria in the hospital shows, however, that the change in the case-mortality was abrupt, and that the introduction of the new treatment was followed by a succession of sixty cases without a single death.

But the most convincing evidence that the fall in the case-mortality of patients treated in the Mile End Hospital was due to acting upon the resolution of the Sanitary Committee that all should have the benefit of antitoxin at the earliest possible moment, and not to a decline in the severity of the disease, is shown by the concurrent mortality of persons treated in their own homes, for upon the case-mortality of these patients the resolution could have had little or no influence.

Amongst the latter the case-mortality was 10·8 per cent. before the resolution (37 cases) and 14·5 after (48 cases) (chart No. II.). From

¹ Eight patients admitted immediately prior to July 16th are included in group II. all of whom had antitoxin previous to admission. The quantity of antitoxin and the date of administration in each of these cases is known to me, and they have been transferred from period I. to period II. in order that a true comparison may be made between the two forms of treatment. Seven others, none of whom died, also received antitoxin before admission, but I have left these in period I. as my information about them is not so exact.

² Even if sixteen persons, notified to have diphtheria, in whom no diphtheria bacilli were found, be deducted, the case-mortality during this period was only 6·6 per cent.

CHART I. Weekly notifications of Patients suffering from Diphtheria at Colchester during 1901.

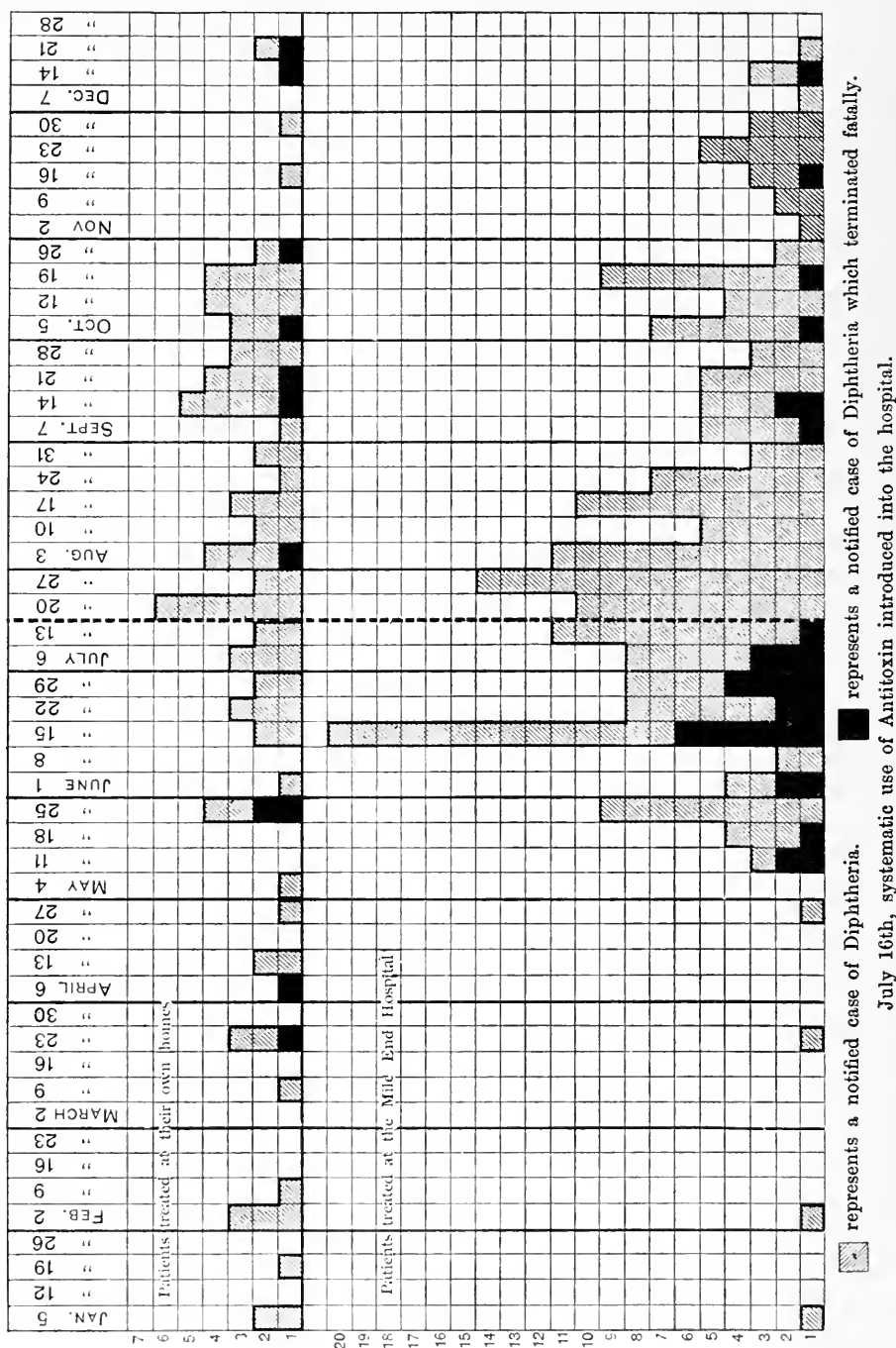


CHART No. II.

	Total		Hospital Patients				Patients treated at home		
	Notifica- tions	Deaths	Admissions	Deaths	Case Mortality		Notifica- tions	Deaths	Case Mortality
January	8	—	2	—	0 %	Mean Case Mortality 25.9 %	6	—	0 %
February	1	—	—	—	—		1	—	0 %
March	5	1	1	—	0 %		4	1	25 %
April	5	1	1	—	0 %		4	1	25 %
May	26	7	20	5	25 %		6	2	33 %
June	46	13	38	13	34 %		8	—	0 %
Before 16th July	27	3	19	3	15 %		8	—	0 %
After 16th	39	1	31	—	0 %	Mean Case Mortality 5.8 %	8	1	12 %
August	38	—	29	—	0 %		9*	—	0 %
September	35	5	22	3	13 %		13*	2	15 %
October	32	4	19	2	10 %		13	2	15 %
November	15	1	13	1	7 %		2	—	0 %
December	8	3	5	1†	20 %		3	2	66 %

* One case in each of these months treated by a private medical man without antitoxin in the hospital and transferred to this column.

† Patient died within one hour of admission. Had been ill several days and received no antitoxin.

these figures it is evident that the severity of the disease did not decline, and it may fairly be concluded that the adoption of the anti-toxin treatment was the means of saving many lives.

Measures taken to check the spread of the disease.

As has been already shown the epidemic began to assume serious proportions in May, and continued to increase till July. The following were the measures adopted up till that month in the hope of checking the spread of the disease.

Patients suffering from diphtheria were during this period isolated either in the Infectious Hospital or in their own homes, but in the majority of cases no bacteriological examinations were made, either with the view of confirming diagnosis, or of determining when convalescents were free from the presence of diphtheria bacilli in their throats.

Members of the families from which the notified persons came, who were attending schools, were excluded from them for a short period. Provided no other person in the house had contracted the disease within a fortnight, the scholars were allowed to return to school after the disinfection of their homes.

It was also deemed advisable to close the public elementary schools about the middle of June, following the notification of twenty-two persons, all school children, during the week ending June 15th.

Towards the end of July the Town Council through Professor G. Sims Woodhead, of Cambridge, invited Dr Louis Cobbett to meet certain members of the Council in order to discuss with them what means should be adopted to check the epidemic, and at their request he put his proposals into writing in the following letter.

PATHOLOGICAL LABORATORY,
CAMBRIDGE,
July 31st, 1901.

To the Town Clerk—for the Chairman of the Public Health Committee.

DEAR SIR,

In accordance with the suggestion which you made at our meeting held in your office this afternoon, I send you the following proposals for dealing with the outbreak of diphtheria in Colchester.

In the first place I would point out that, in the absence of evidence of the propagation of the disease in your town by milk or infected animals, it is most reasonable to conclude that it is being spread by personal contact.

In this connection it must be remembered that those who suffer from recognised diphtheria are not the only sources of infection. Quite as important are those who suffer from so mild an attack that medical advice is not sought, and those who having come in contact with cases acquire the bacillus, and carry it in their throats and noses without any illness whatever. Indeed I think that those who belong to these last two categories are more responsible for spreading the disease than are the notified cases, because no precautions in regard to them are usually taken.

It is therefore of first importance to discover as many of these persons as is possible, and that without delay, and this can be done by bacteriological examination. Such infected persons when found should be isolated until they have been proved free from infection. Seeing that they are only to be found among those who have come into intimate contact with cases of the disease or with others infected like themselves, it ought not to be difficult to determine what persons should be examined.

In order to limit the necessary amount of work involved in carrying out this proposal, it is desirable that the diagnosis of all notified cases should be founded in part on bacteriological evidence; for experience in the London Metropolitan Hospitals and elsewhere has shown that from a quarter to a third of all cases notified as diphtheria on clinical evidence alone are not really instances of that disease, but suffer from membranous sore-throat of another kind.

Secondly, the very beneficial results obtained recently in Cambridge from prophylactic injections of 500 units of antitoxin given to those who have come in contact with cases of diphtheria induce me to strongly recommend this measure also.

Now as to the means of carrying out these proposals I would recommend that:—

(1) A practical Bacteriologist be appointed to make the necessary examinations, and that he be provided with a suitable laboratory and laboratory servant. (While this is being arranged swabs might be sent to Cambridge and examined there.)

(2) That a house and garden be taken, put in charge of a trained nurse, and opened as an Isolation Home for healthy infected persons. (The house might be situated on the outskirts of the town.) All persons admitted to the Home should have a prophylactic injection of antitoxin.

(3) That a circular letter be sent to all the private medical practitioners,

(a) Advising them of what it is intended to do.

(b) Offering them bacteriological examination for their poorer patients free of charge, as well as a free supply of antitoxin.

(c) Requesting them to submit swabs from all cases which are in the least suspicious, as well as from those which they notify; and from all children and young persons living in houses where these cases occur. (I do not think that the parents themselves should be examined, but would suggest that they should be instructed to make use of some antiseptic throat-wash night and morning.)

(d) Informing them that if they do not wish to be troubled with the examination of healthy "Contacts" the Medical Officer of Health is prepared to undertake this himself and to give prophylactic doses of antitoxin. The notification form might be made to contain a space in which the medical practitioner could state whether he wished the Medical Officer of Health to undertake this work.

(e) And finally requesting them not to regard their patients as free from

infection after a certain lapse of time, but only after bacteriological examination has been made with three consecutive negative results.

I should also suggest that the Medical Officer of Health seek out, and cause to be bacteriologically examined, all persons whom he thinks likely to have come in contact with infection, or cases of suspicious sore-throat of which he may be informed by his inspectors and others, where no medical man is in attendance. In this work he would probably require assistance, which might be afforded by a young qualified medical man.

It is evident that to carry out these measures it will be necessary to have the cordial cooperation of the medical profession and the public. It has been our experience at Cambridge that with patience and tact it is possible to induce parents to consent to the desirable measures.

I am, Sir, yours truly,

(Signed) LOUIS COBBETT, M.D., F.R.C.S.

This letter was read at a meeting of the Sanitary Committee held on August 9th, and after a careful and lengthy discussion the proposals were adopted with the exception of the provision of an Isolation Home, and Dr Cobbett was given the authority necessary to put these proposals into practice.

In the above letter to the Sanitary Committee Dr Cobbett points out that in his opinion diphtheria is very largely spread either by persons suffering from so mild an attack that medical advice is not sought, or by persons who harbour diphtheria bacilli in their throats and yet remain perfectly well. In other communications he specified some of the ways in which the two classes of persons just described, and more especially the children amongst them, disseminate the disease. He gave as examples, the custom of allowing two or more children to sleep in one bed; their congregation at school; the fact that their toys, pencils, and school implements freely pass from one to another; and finally their habit of putting these and other things into their mouths. In explanation of why infected persons may remain well, he quoted the fact that about 50 per cent. of healthy persons have diphtheria antitoxin in their blood.

During the outbreaks of diphtheria which occurred in Cambridge in the Autumn of 1900, and Spring of 1901, he was in a position to test the soundness of these views by finding and isolating such persons as he considered to be spreading the disease. The results gave some degree of justification for considering that his opinions were correct and the measures he adopted of practical value¹.

¹ See *Journal of Hygiene*, vol. 1., no. 2.

All the measures which he suggested for checking the outbreak at Colchester were based on these observations, and the considerable degree of success achieved is additional evidence in support of his view.

Experiments made at the Mile End Hospital and at the laboratory add further confirmative evidence of the correctness of his theory. Plate cultures were exposed in the wards of the hospital in various positions, but failed to demonstrate the diphtheria bacillus in the air of the wards. Similar experiments were made in the laboratory with like results.

The application of the measures proposed by Dr Cobbett.

On August 15th a circular letter was forwarded to all the medical practitioners in the borough in the terms suggested in section 3 of Dr Cobbett's letter to the Sanitary Committee, and by August 21st¹ a laboratory had been fitted up in Dr Chichester's house and work was commenced there.

As a result of these measures a considerable number of swabs were submitted for examination by the resident practitioners, and the diphtheria patients in the Mile End Hospital were bacteriologically examined, and none discharged till three consecutive negative examinations had shown them to be free from bacilli.

In accordance with these plans all patients admitted since July 16th had been detained in the hospital in order that their throats should be bacteriologically examined, and by these means it was discovered that several of them, who by this time were apparently well, still harboured diphtheria bacilli in their tonsils. Although the majority of these soon became free from their bacilli, and were discharged, two retained them for nearly twelve weeks longer, despite continuous efforts to destroy the parasites by the application of antiseptics.

Apart from the patients already admitted into the hospital a few persons, who had been treated in their own homes, were found to be in a similar condition, and some patients with suspicious sore throats were proved to be suffering from diphtheria, whilst in the case of others it was shown that the specific organisms of diphtheria were not present.

Mention has already been made in the foregoing history of the epidemic of the fact that the first fall in the notification rate per month occurred during August, and that the level of this month was maintained during September and October. This decrease in the number

¹ Between August 14th and 21st swabs were sent to Cambridge for examination.

of the cases notified, and more especially the continuance of the lowered notification level during months which are commonly the worst for diphtheria, may, I think, be fairly ascribed to the measures employed.

During the first few weeks the work was almost confined to the examinations described, but while they were being conducted a system for dealing with current cases as they arose, and with those who came in contact with them, was gradually being evolved, which, however, did not reach its ultimate form till after the examination of the school children had been completed. Details of the course adopted in connection with these cases will be fully explained presently.

Re-opening of the Public Elementary Schools.

The public elementary schools had been closed since the middle of June, and owing to the diminution in the notification rate it was decided to reopen them on September 9th. At the meeting of the Sanitary Committee, in which this step was decided on, a resolution was also passed instructing me to examine all the scholars bacteriologically. At the next meeting I pointed out that owing to their number (6,000) this could not be done in any reasonable time, and Dr Cobbett wrote to the Chairman showing with what a risk of increasing the prevalence of diphtheria the opening of the schools would be attended unless measures were taken to exclude the infected children.

As a result of these communications it was arranged that: (i) those schools in which diphtheria had chiefly occurred during the last few months should be kept closed till September 29th, and that the others should be opened on September 15th. (These latter accommodated rather less than half of the school children of Colchester.)

(ii) Those children living in houses in which a case of diphtheria had occurred later than June 1st should be excluded from school until they had been ascertained by bacteriological examination to be free from diphtheria bacilli.

The following means were used for carrying out the work.

With the aid of Mr Wells, the Inspector of Nuisances, a list was made of children who lived in houses in which a case of diphtheria had been notified since June 1st, and these children were classified according to the schools which they attended. With the concurrence of the school authorities the various head-masters and mistresses were notified by letter on no account to admit children whose names were on the

lists until they had received a notice stating that bacteriological examination had shown them to be free from diphtheria bacilli. The parents were also notified of this decision by letter, and were requested to send their children up to Dr Chichester's surgery for the necessary examinations. This invitation was well responded to, the parents being anxious that their children should return to school. In a few instances only was it necessary for the Sanitary Inspector to make a personal visit, and remind parents that the decision would be strictly adhered to.

Further, a meeting of the head-masters and mistresses was called to discuss with Dr Cobbett the best means of dealing with the pens, pencils, slates, and other school implements.

As a result of this conference the school teachers became informed about the possible ways in which diphtheria bacilli might be passed from one child to another, and measures were adopted for insuring that each child should have its own pens, pencils, and slate, and for the systematic disinfection of these articles. The teachers were also requested to send notice to the laboratory of any cases of sore-throat or other suspicious illness which might come to their notice.

As an additional precaution it was decided to remove the cups from the public drinking fountains, and a little later the water was cut off in order to prevent the children drinking from the spouts.

The work of examining the suspected persons was necessarily heavy and was finally completed about October 15th.

Four hundred and seven scholars from nineteen schools were examined as well as fifty-nine persons either above or below school age, members of the families to which the school children belonged, and fifty-three children from the Colchester Union, making in all five hundred and nineteen persons, from whom 861 swabs were obtained. Of these 519 persons 54 (10·4 per cent.) were found to be harbouring diphtheria bacilli in their throats, though they themselves remained in perfect health.

All these infected children together with the other members of their families of school age were excluded from school.

Owing to the stress of work some of these were not again examined for three weeks, but at the earliest opportunity all were re-examined, and if found free from diphtheria bacilli were, after three negative examinations, allowed to return to school.

On October 15th there remained fifteen of these children, who still retained diphtheria bacilli, and all were treated daily at Dr Chichester's surgery by means of antiseptics applied to their throats. Although in

a few days the children could tolerate the application of strong antiseptics to their tonsils, the treatment was not found to be very successful in freeing them from their bacilli, but was thought to render them less liable to communicate the bacilli to others.

On November 14th six remained and these were admitted to the Isolation Home (see below).

Since the Autumn and early Winter are as an almost universal rule by far the worst times for diphtheria, the outlook during September was very disquieting, and the opening of the schools was watched with anxiety, the more so as the mean notification rate was still eight per week. In spite of these adverse conditions the results of the measures just described were highly satisfactory, and lend very strong support to the view held by Dr Cobbett that diphtheria is largely spread by healthy persons harbouring diphtheria bacilli in their throats.

Chart No. III. has been constructed to illustrate the results of these measures upon the dissemination of diphtheria amongst the school children. It shows that after the completion of the examinations on October 19th, and the exclusion from school of the healthy children harbouring diphtheria bacilli found in the course of them, no case of diphtheria was notified amongst the scholars except from the Barrack Street, St Mary's, and Kendall Road Schools.

In the case of Barrack Street School a period of eight weeks elapsed and three cases were then notified. (One died before any cultivations could be taken, and no diphtheria bacilli were found in the other two patients.)

In St Mary's School two cases occurred during the week ending November 23rd, both confirmed by bacteriological examination.

At the Kendall Road School, on the other hand, there was a small outbreak extending over four weeks, in the course of which seven cases were notified.

These patients were all scholars in the Infants' department. The children attending this department (112) were all bacteriologically examined on November 30th, and five of them, harbouring diphtheria bacilli, were excluded from school.

Since that date no further case has been notified from the Kendall Road School.

CHART No. III.

Half the schools { opened

* re-examination of Kendall Road School completed.

Measures adopted for dealing with those who had been in Contact with Fresh Cases.

While the arrears of the past three months were being worked off the method of dealing with current cases was gradually organised.

Notifications of diphtheria were received at the laboratory. As each notification came to hand the Inspector was sent to the house in which the patient was living to make a list of all the inmates, their ages, and schools, if any, which they attended. He was also instructed to inquire whether any other children were intimate with the patient and to add their names to the list.

The parents of all these children were informed that none of them would be allowed to go to school until they had been bacteriologically examined, and all the children then in the house had been found to be free from infection; and they were requested to bring their children to Dr Chichester's surgery to be examined. In some cases, however, the necessary swabs were obtained through the doctors in attendance.

Notices similar to those already described were sent to the schoolmasters and mistresses concerned, warning them not to receive the children until further notice.

Prior to the opening of the Isolation Home, when a child harbouring diphtheria bacilli was discovered all members of the family of school age were excluded from school, but subsequently this was only done when the parents refused to allow the infected child to be isolated.

The Isolation Home.

At a meeting of the Sanitary Committee held on September 18th, the question of isolating healthy "contacts" who were found to harbour diphtheria bacilli in their mouths was again raised, and a list of twenty-five persons was submitted. It was thereupon resolved to refer the question to a special meeting to be held on September 25th.

On that day the Sanitary Committee unanimously resolved to obtain a house wherein such persons could be isolated and treated, and the Borough Surveyor was instructed to take steps to obtain Severall's Hall for the purpose, a farm-house with a considerable amount of ground suitable for the recreation of the inmates, situated about two and a half miles from the centre of the town.

This house was obtained and put in order, but owing to its requiring many repairs was not ready for the reception of infected persons till

November 14th. It contains two sitting-rooms for isolated persons and bedroom accommodation for fifteen, besides accommodation for a matron, nurse, and two servants.

Owing to the delay in opening the Home the infected children discovered early could not be isolated. On November 14th only six of these children still remained infected, and these were accordingly admitted.

Three "contacts" with more recent cases were also received, but the parents' consent to the isolation of three others could not be obtained.

Under the circumstances the Isolation Home could not have played a very important part in bringing the epidemic to an end, though it will doubtless be of great value in the future. Better results might have been obtained had the Isolation Home been available for the healthy "contacts" at an earlier period.

The most effective step actually taken was probably the exclusion of the infected children from the schools, and the constant application to their throats of antiseptics, which though it seemed to have but slight influence in freeing them from their bacilli, probably made them less dangerous to others than would otherwise have been the case.

From the foregoing account it is seen that the parents of infected children, and other healthy adults, who might have come in contact with cases of diphtheria, were not systematically examined. This course was adopted, firstly, because it was thought impracticable to isolate those who were earning the living of the family; and, secondly, because if it had become known that certain persons, in whom diphtheria bacilli had been found, were not isolated, it might have led to difficulties in procuring the isolation of children who, it cannot be doubted, are from the nature of their habits and the circumstances of their lives more liable than adults to convey to others any micro-organisms that they may have in their mouths (see p. 178).

Decline of the Epidemic.

In November the recorded cases fell from thirty-six, the mean rate of the previous three months, to fifteen, and this was followed by a further decline in December, when only eight persons were notified as suffering from diphtheria, up to the 28th.

Of these twenty-three notified persons eighteen were treated in the Infectious Hospital, but in seven of the latter the diagnosis was not

confirmed by bacteriological examination, though several cultures were obtained from each of them.

Including these persons the mean weekly notification rate of the last nine weeks of the year was 2.5, as against 8 in the previous nine, and 13 in the eleven weeks preceding the latter.

Finally on December 28th only two patients, both convalescent, remained in the hospital and two in the Isolation Home.

The decline in the epidemic followed so closely on the measures for dealing with the scholars of the public elementary schools that it can scarcely be doubted that it was brought about by these measures, and I consider that there is good reason to think that if these precautions had been employed in May and June, when the area in which the disease prevailed was limited, the further spread of the outbreak might have been considerably reduced.

The Bacteriological Examinations.

The carrying out of these measures involved the bacteriological examinations of 1891 swabs, 693 from the hospital, 337 from general practitioners, and 861 obtained directly from school children and others whom it was thought desirable to examine on account of their having come more or less closely in contact with actual cases of diphtheria.

Diphtheria bacilli were found in 436 cultivations (23 per cent.).

On the subject of the pseudo-diphtheria or Hofmann's bacillus the observations made at Colchester are in accordance with Cobbett's views on this organism.

As the result of his investigations at Cambridge¹ in 1900 and 1901 he came to the following conclusions:—

(1) That the experience of the outbreak at Cambridge gave no reason for thinking that the pseudo-diphtheria bacillus is other than perfectly innocuous to man.

(2) That the frequent presence of the pseudo-diphtheria bacillus should not be allowed to weaken our efforts to detect and isolate those who harbour the virulent bacillus.

His observations during the Spring outbreak at Cambridge², of 1901, tended to confirm his belief in the opinions just quoted.

¹ "The result of 950 bacteriological examinations for diphtheria bacilli during an outbreak of diphtheria at Cambridge and Chesterton." Louis Cobbett, M.D., F.R.C.S., *Journal of Hygiene*, vol. 1., p. 258.

² "Observations on the recurrence of diphtheria at Cambridge in the Spring of 1901." Louis Cobbett, M.D., F.R.C.S., *Journal of Hygiene*, vol. 1., p. 494.

At Colchester, although full particulars of all the micro-organisms found are not enumerated in a few instances, the pseudo-diphtheria bacillus is recorded as present on 586 occasions, or 31 per cent., of all swabs examined—and amongst the 112 scholars of the Infants' department of the Kendall Road School, 66 or 59 per cent. harboured this bacillus.

From these figures it can be seen how commonly this bacillus made its appearance in the cultures, but it probably occurred even more commonly than is here represented, for in 157 cultures only the presence or absence of diphtheria bacilli is noted, and in many others when diphtheria bacilli have once been found no further search for the pseudo-diphtheria bacillus was made.

Further, it was noticed in this epidemic, as it had been at Cambridge, that the pseudo-diphtheria bacillus was most frequently found in the throats of the children of the poorer classes, and also that it seldom, if ever, appeared to exercise any influence on the person in whom it was discovered.

The Persistence of Diphtheria Bacilli in the throat.

Following the practice adopted at Cambridge and other places¹ it was decided that a patient should only be considered free from infection after three consecutive negative examinations, and the following facts appear to indicate that this course gives a practical measure of safety, if the last two examinations are made after all local antiseptic treatment has been discontinued².

Of the patients discharged from the hospital during period II. thirty were re-examined on subsequent dates and all but three³ were found to be free, and, moreover, in no case, with one doubtful exception,

¹ At the South-Western Fever Hospital the patient is detained till the bacilli disappear as evidenced by three consecutive daily examinations. *Guy's Hospital Gazette*, vol. xv., p. 294.

The Boston Board of Health, U.S.A., require for hospital patients three consecutive negatives. Paper by H. W. Hill, M.D., *Journal of the Massachusetts Association of Boards of Health*, vol. viii., Oct. '98.

² Antiseptic applications, if any were being used, were not applied within the twenty-four hours previous to a swab being taken. If the result of the examination showed that diphtheria bacilli were no longer present treatment was entirely discontinued (unless they occurred on a subsequent occasion) in order that the presence of antiseptics on the swab might not hinder the growth of the organisms on the culture media.

³ One of these suffered from a second well-marked attack of diphtheria and another had been in contact with a recent case immediately previous to examination.

was it brought to my notice that a discharged patient was an agent in spreading infection.

Amongst the hospital patients, if a few exceptional cases are excluded, the mean duration of the period during which the diphtheria bacilli were found to persist was 28 days from the date of notification, but in some of the exceptional cases they lingered up to 87 days.

A few of the healthy children, found to be harbouring diphtheria bacilli, also retained them for long periods, in one case up to 94 days.

A small proportion of these persons, in whose throats the bacilli obstinately remained, had very large tonsils, but in the others no abnormal conditions could be found to account for their long persistence.

Ages of Persons notified to be suffering from Diphtheria.

Between January 1st and December 28th 285 persons were notified, of whom 77 were above, and 208 below, fifteen years of age. 72·9 per cent. of all notified persons were consequently below the age of fifteen.

In 1900 the proportion of patients treated in the infectious hospital over, to under, fifteen years was about the same; 71·4 per cent. being under that age.

These figures afford an explanation of the marked fall in the notification rate which occurred when the disease was almost stamped out amongst the children of school age.

It is also interesting to note that after this occurrence not only did the notifications decline, but a decided change took place in the proportion of adults to children notified, as is shown in the following table.

	Persons notified		
	Total	Above 15	Below 15
Week ending October 19th	13	7	6
" " " 26th	5	2	3
" " November 2nd	1	1	—
" " " 9th	2	—	2
" " " 16th	4	1	3
" " " 23rd	5	2	3
" " " 30th	4	2	2
" " December 7th	1	1	—
" " " 14th	4	1	3
" " " 21st	3	2	1
" " " 28th	—	—	—
	42	19 (45 $\frac{1}{10}$)	23 (54 $\frac{1}{10}$)

Up to the week ending October 12th, 75·6 per cent. of notified persons were under fifteen years of age.

The Case-mortality of Patients above, and below, fifteen years of age.

During the year 36 of the 208 persons under fifteen years of age notified to be suffering from diphtheria died (17·31 per cent.) and only three of the 77 above that age (3·9 per cent.); consequently the mortality amongst the children was nearly four and a half times as great as it was amongst adults.

At the Mile End Hospital the percentage case-mortality for each class was much higher in period I. than in period II. The records for period I. (p. 171) show that twenty persons under, and one over, fifteen years of age, died, making the case-mortalities 27·4 per cent. and 11·1 per cent. in each class respectively.

With the introduction of the systematic use of antitoxin during period II. a very marked reduction in the case-mortalities became apparent, for while no person over fifteen died, only seven deaths of patients under that age occurred, the resulting case-mortalities for each class being 0 and 9·09 per cent.

From August till the end of December Dr Louis Cobbett acted as Consulting Bacteriologist to the Town Council, and every measure adopted was in accordance with his proposals. On several occasions also he was present and assisted in the practical part of the work.

Dr E. Chichester, in whose house the laboratory was established, not only took all the swabs from the hospital patients, but also very kindly allowed us to make use of his surgery for examining, swabbing, and treating contacts and other persons. Throughout he rendered every assistance in his power, and especially offered valuable suggestions as to the local treatment of infected throats, which he put into practice on hospital patients who obstinately retained their bacilli.

At a meeting held on December 18th the Sanitary Committee decided to appoint Dr Chichester to carry on the work on the lines indicated.

Dr J. R. Watson also rendered great assistance at the time when the school children were being examined and the pressure of work was very great.

To Mr Wells, the Inspector of Nuisances, a great part of the credit for the effectual application of these precautionary measures is due. From the outset he thoroughly grasped the principles on which the work was being conducted and brought to bear on the part intrusted to him great tact and energy.

Summary.

Finally I wish to draw particular attention to the following facts:—

1. The striking diminution in the case-mortality at the Mile End Hospital which followed the systematic use of antitoxin.
2. The subsidence of the epidemic during the season when diphtheria is commonly most prevalent.
3. The opening of the schools in September without any increase in the prevalence of the disease.
4. The persistence of diphtheria bacilli for long periods in certain of the convalescents and contacts.
5. The success of preventive measures based upon the belief that diphtheria is spread mainly by personal contact, and through the intermediation of healthy persons and others who by the means usually employed are not recognised to be suffering from the disease.

ADDENDUM.

Considering the extent to which the outbreak had spread it could scarcely be hoped that we had succeeded in finding every person harbouring diphtheria bacilli even amongst the scholars of the elementary schools, and consequently I was not surprised to learn that several fresh cases of diphtheria had been notified in the early part of the year 1902.

Between December 28th and February 23rd twenty-seven patients have been notified, the weekly returns being 7 during the week ending January 4th, and 4, 5, 2, 5, 3, 1 in the weeks following.

Fifteen of these were either scholars of Barrack Street School or persons connected with them, and five were connected with the Culver Street or Wesleyan School, and the main part, if not the whole of the outbreak in these two schools, can, I think, be traced to the neglect of bacteriological examinations in two instances.

Chart IV. has been constructed to show the lines along which I am inclined to think the specific organisms were carried to the various persons in connection with these schools who developed the disease.

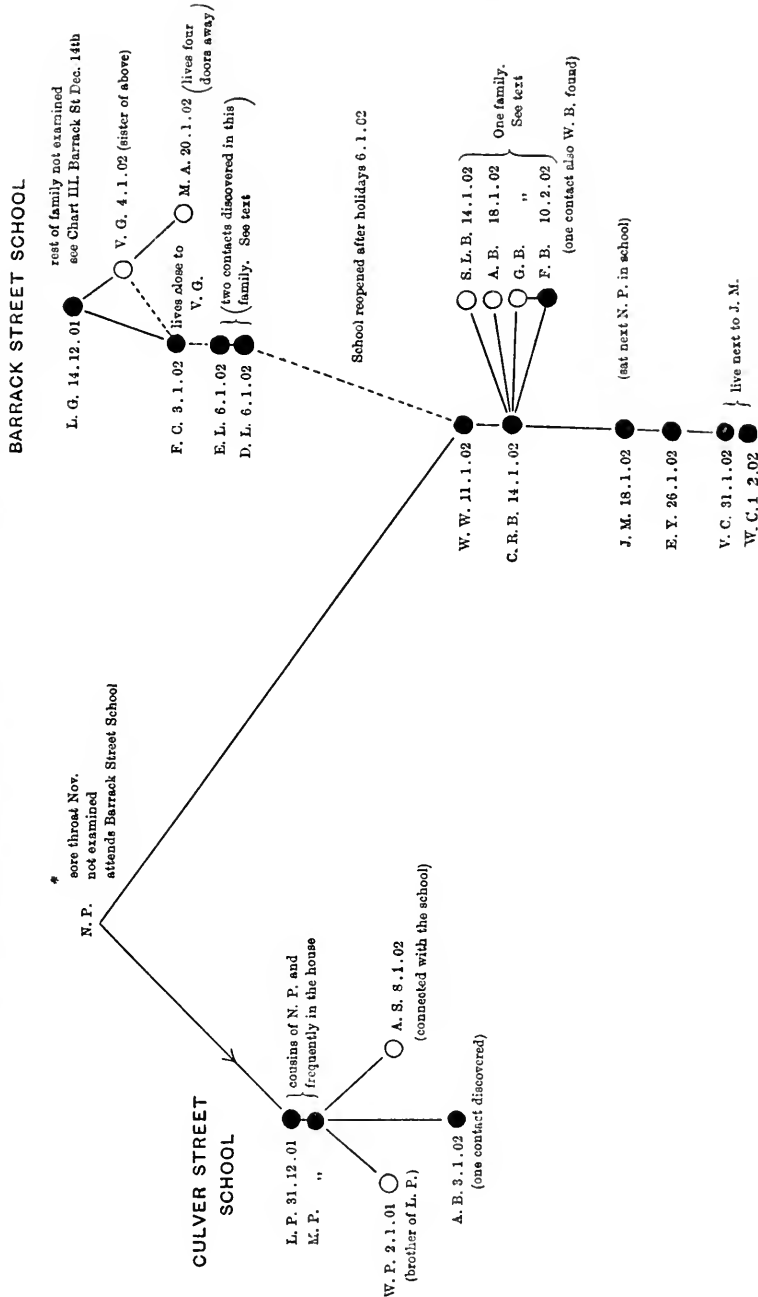
The child mainly responsible for the outbreak I believe to be N. P., and L. G. seems to have been also an agent in spreading the disease.

Neither was proved by bacteriological examination to have diphtheria bacilli in her throat. N. P. was not examined because at first no information concerning her reached me, and later because her parents refused to allow a swab to be taken. L. G. died before any examination could be made.

N. P., who was a scholar of Barrack Street School, suffered from a sore throat in the early part of November, which was considered by the neighbours to be diphtheria, but as stated, no bacteriological examination was made. This child

CHART IV.

Children attending the Barrack Street and Culver Street Schools, and others in connection with them, notified to have diphtheria since December 28th.



* N. P. returned to school on 6. 1. 02, when it reopened after the holidays, and attended till 14. 1. 02 when she was excluded. Parents consent to examination could not be obtained.

• signifies a scholar notified to have diphtheria.

Initials and dates of notification given for each case. Straight line indicates probable line of infection, directly or through intermediate contacts; dotted line possible line of infection.

○ signifies other persons connected with scholar.

remained at home till January 6th when the school reopened after the holidays, and then she again commenced to attend and continued to do so till January 14th.

L. P. and M. P., cousins of the above child, were frequently in the house, and on December 31st were notified to be suffering from diphtheria. Both were scholars of the Culver Street School and continued to attend until the commencement of the school holidays immediately before Christmas.

These two were the first cases recorded in this school since September 14th.

Subsequently their infant brother was attacked and two other persons, one connected with the school and the other a scholar, and one healthy contact was found in connection with these cases. No further cases occurred at this school.

At the Barrack Street School ten scholars suffered from diphtheria and five persons apparently connected with them.

Three scholars and two others were notified before the opening of the school and the return of N. P. These seem to be connected with the case of L. G., a scholar, who was notified as suffering from diphtheria on December 14th (Chart III.) and died before a swab could be obtained. That the disease from which she suffered was diphtheria appears to be certain from the fact that her sister V. G., who was not a scholar, was notified on January 4th. These two seem to have communicated the disease to F. C. and M. A., children who lived close to them, the former being a member of Barrack Street School.

On January 6th two children, both members of the same family, and scholars of this school, were notified, and two contacts were found in the family, who may have been responsible for passing the disease from L. G. to the other two members of their family.

This small epidemic shown on the Chart IV. in the right upper corner, appears to have arisen from the case of L. G. and was not I think connected with the outbreak after the reopening of the school, although some unknown contacts with these cases may have been instrumental in handing on the disease to some of the patients subsequently notified.

N. P., who seems to have been the original cause of the outbreak in the Culver Street School, returned to the Barrack Street School when it reopened after the holidays on January 6th and continued to attend till the 14th, when she was excluded.

Five days after the opening of the school a scholar, W. W., was notified and three days later another, C. R. B. Shortly afterwards J. M., who had been sitting next N. P., developed the disease, and subsequently two children who lived in the next house to her. The course of these events can be followed in the diagram.

All the children notified are not members of the same class, but all meet in the playground and cloakroom.

The small outbreak in the Culver Street School evidently started by L. P. and M. P., who were frequently in contact with N. P., combined with the fact that N. P. suffered from a sore throat of such a nature that the neighbours considered it to be diphtheria, and which kept the child away from school for two months, seem to point to the disease from which N. P. suffered being diphtheria. Additional weight is lent to this view by the spread of diphtheria at Barrack Street School when she again attended as a scholar, and also by the fact that the outbreak came to an end about a fortnight after her exclusion from school, and that the school has remained free for three weeks afterwards (date of writing).

These arguments might have been much strengthened had it been possible to obtain the parents' consent to the examination of her throat, but in the absence of bacteriological evidence, the several facts stated suggest that she was one of those patients who suffer from a mild attack and afterwards harbour the bacilli for a long time.

The case of C. R. B. is of especial interest, for by the use of the bacteriological test the further spread of the disease by means of this family was probably prevented.

C. R. B. and S. L. B. were notified on January 14th and diphtheria bacilli were found in the throats of four other members of the family. The throats of all four appeared normal and they complained of no illness. No antitoxin was given, but they were isolated. In the course of a few days two of them developed the disease, and one three weeks later.

Of the seven remaining cases one is particularly noteworthy. A. S., the sister of R. S., was notified on August 6th and was treated in the hospital. On September 19th, in the course of the examination of the infected scholars, R. S. was found to be harbouring diphtheria bacilli. He was isolated and treated until December 15th, when he appeared to be free from bacilli and was sent home. On January 14th he was notified to be suffering from diphtheria. This is the only case recorded in the St John's Green School between October 19th and February 23rd.

Two cases occurred amongst the nurses in the hospital, who probably derived their bacilli directly from the patients.

I can offer no explanation as to the origin of the last four cases, having received no information about them apart from the statement that they attended certain schools, nor do I know whether the diagnosis was confirmed by bacteriological examination.

One notified on December 30th attended the Kendall Road School, one Lexden School, one Shrubbs End School and one Greenstead School.

The last three schools are placed in Chart III. under the heading "Other Schools." All are situated on the outskirts of the town and in none of them had a case been previously notified during the year.

This recrudescence of the disease has consequently been almost confined to the Barrack Street and Culver Street Schools; and every other school within the town, with the exception of Kendall Road, with a small outbreak in November and one case later, and St John's Green with one case, have remained free from October 19th, the date of the completion of the examination of the infected children, till the time of writing (February 25th) a period of eighteen weeks.

Of the 27 persons notified, 15 can apparently be traced to N. P., 5 to L. G., and 3 also of the remaining 7 can be accounted for.

Dr Chichester has most kindly furnished me with the results of many of his bacteriological examinations, and Mr Wells has supplied me with much information about all these cases, which must have cost him much time and trouble to procure.

EXPERIMENTS ON THE RELATION OF THE COW TO MILK-DIPHTHERIA.

(Plate II.: Seven Figures.)

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THE present communication relates to an outbreak of diphtheria where suspicion was directed to the milk supply and where there existed a pathological condition of the udder of the cows from which the milk was obtained.

There has been in Great Britain for many years a prevailing impression among sanitarians that milk is capable of acting as a vehicle of infection in diphtheria. Indeed there is a mass of evidence connecting certain epidemics with the milk supply.

Before the discovery of the specific organism of diphtheria, Mr Power had pointed out in his Report to the Local Government Board in 1878 the connection between the disease and the milk supply in an epidemic investigated by him in North London. He was led by a process of exclusion to the surmise that actual cow conditions capable of affecting directly or indirectly the milk might have brought about the results observed.

A number of more recent epidemics which were carefully investigated,

Devonport, 1882,—Dr Parsons ⁽¹⁾,

Hendon, 1888,—Mr May ⁽²⁾,

Hendon, 1882,—Mr Power ⁽³⁾,

York Town and Camberley, 1886,—Mr Power ⁽⁴⁾,

have emphasised the connection between milk supply and diphtheria.

Howard ⁽⁵⁾ in America has carefully worked out an epidemic in which milk appeared to be the vehicle of infection. In his paper he points out that Escherich has called attention to the fact that, outside of England, no milk epidemics of diphtheria have been reported.

Though a number of diphtheria-like bacilli have been demonstrated in milk, in very few instances has the presence of a virulent organism been recorded.

Bowhill ⁽⁶⁾, in connection with an outbreak of diphtheria at Cardiff, attributed to infected milk, isolated from the suspected milk, a diphtheria bacillus whose virulence for guinea-pigs was proved by Dr Nuttall.

Eyre ⁽⁷⁾ and Klein ⁽⁸⁾ have also recorded the existence of a genuine virulent diphtheria bacillus in milk.

It has always been a question of doubt as to whether the infective material is derived from the cow herself, or from subsequent contamination of the milk by human or other agencies. As stated above Mr Power was inclined to regard the cow as the source of infection.

In several epidemics lesions have been observed in the udders of cows yielding the suspected milk; notably in the outbreaks at Devonport in 1888 ⁽²⁾ and York Town in 1886 ⁽⁴⁾; and Dr Thorne Thorne (1891, p. 192), in reviewing the chief epidemics of "milk-diphtheria" in England, sums up as follows:—

"On each occasion of milk-diphtheria to which I have referred there has been evidence, more or less precise, of some cow-ailment, so far trivial, it is true, as to be ignored by those versed in bovine diseases, but either affecting the physical properties of the milk, or being associated with some vesiculation, and later on with 'chapping' or 'scabbing' of the udder and the teats."

In those conditions of the udder which have occurred apart from artificial infection, so far as we are aware, the *Bacillus diphtherie* has not hitherto been demonstrated.

Klein ⁽⁸⁾, following Power's observations, attacked the question from the experimental standpoint. He inoculated recently calved cows with cultivations of *B. diphtherie* of human origin. He found that these cows "became attacked by a definite illness, having, as one of its manifestations, a peculiar acute eruptive infection of the udder; and that from the milk of an animal made ill in this way the diphtheria bacillus could be isolated by cultivation." There was present also in the animals disease of the lungs, liver and kidneys. All these phenomena he attributed to one cause, viz., the inoculation with the diph-

theria bacillus. The eruption on the udder was not invariably present, nor could the bacilli be always demonstrated in the milk. In a later communication ⁽¹⁰⁾ he pointed out that in two outbreaks of diphtheria, one near Croydon, the other near Bishop's Stortford, lesions occurred in the udders of the suspected cows having the same characters as those produced by experimental inoculation. He did not claim, however, to have demonstrated bacteriologically either the diphtheritic character of the lesions or the presence of the bacillus in the milk. Loeffler ⁽¹¹⁾ criticised Klein's results and held that they required further confirmation. Abbott ⁽¹²⁾ attempted to reproduce in two cows the results obtained by Klein. He succeeded in demonstrating the bacilli at the site of inoculation, but failed to obtain cultivations both from the local lesions and from the milk. In his cows there was no eruption on the udder, nor did he find visceral lesions. Klein ⁽¹³⁾ replied to Abbott's criticism and attempted to show the reason why the results differed from his own. Ritter ⁽¹⁴⁾ also attempting to repeat Klein's experiments failed to show that the diphtheria bacilli passed into the milk.

History of Outbreak.

Our attention was drawn to the present outbreak by Mr Sidney Villar, F.R.C.V.S., who had, in the course of his practice, seen two cows suffering from "cow-pox" and who had heard of the existence of sore throats amongst the consumers of the milk.

The two cows were the property of a gentleman, the milk being used chiefly for his family and servants: any surplus being sold to one individual in the district, who distributed it to a few customers.

The milk from the cows was supplied to Mr F.'s family, consisting of Mr and Mrs F., a baby, a nurse, and two other servants in the house. Of these, Mr F. did not drink milk; the baby and nurse drank the milk only after sterilisation.

Of the members of the household drinking unsterilised milk, Mrs F. had typical diphtheria, first observed on Dec. 11th; one of the two servants had a suspicious sore throat and was seen by the family medical practitioner; but as both servants left the house on Mrs F. becoming ill they escaped further observation.

The gardener, who acted as cowman, and lived near the cowhouse, about a quarter of a mile from Mr F.'s house, had a family consisting of a wife and seven children; and a small quantity of the milk went to this household. One of the children, aged four, was removed to the isolation

hospital on December 11th, with a severe attack of diphtheria, necessitating subsequent tracheotomy. Two cases of sore throat among other consumers of the milk were known to Mr F.

On visiting the place on December 15th we found that the two cows were housed in a small cow-house with a low ceiling, and that they had been turned into an adjoining paddock, with two heifers, their own calves of the previous spring. So far as was known there had been no recent contact with other cattle, either directly or through the cowman. Both cows were in calf. The date of the onset of the disease in the cows could not be accurately determined, but the cowman stated that he had observed something wrong with the teats for a week or ten days.

Appearances observed in the Cows on December 15th.

On the udders and teats of both cows there were present papules and ulcers covered by dark brown scabs (see Plate II, Fig. 1). The papules were, on an average, about the size of a pea, and had a markedly indurated base which extended into the subcutaneous tissue. No vesicles were seen at this examination. In the cow which was experimentally infected at a later period and where precautions were taken to avoid their being broken, vesicles were a very marked feature of the condition, as will be seen in Figs. 2 and 3. The majority of the lesions were in the form of ulcers covered with dry brown crusts and were of the size of a sixpence to a shilling. On removing the crust from one of these there was exposed a slightly moist, fairly smooth surface with an elevated, puckered, cicatricial-looking margin. The largest lesion at this stage, situated on the udder itself, measured about 2 inches by 1 inch, had an irregular margin and appeared to have been formed by the running together of several smaller ulcers. The ulcerative process in this case was considerable, and on removing the crust there was exposed a raw, bleeding, irregular cavity with a depth of at least $\frac{1}{4}$ inch. The severity and persistence of the lesions were no doubt due to the constant removal of the crusts either by the hands of the milker or by the animal lying on, and crushing, the udder.

In Cow 1 there was no evidence of mammitis at this stage; there was an abundant secretion of apparently normal milk and the general health appeared to be little, if at all, affected.

In Cow 2, in addition to the surface lesion of teats and udders, there was a distinct mammitis affecting a posterior quarter of the udder. In

this case the milk was scanty, ropy, semipurulent looking, and was slightly tinged with blood. Shortly after removal to the new station this cow cast her calf.

Bacteriological Examination of Material from Cows.

Cultivations in broth, agar, and solidified blood-serum were made from the ulcers after removal of the crusts, and at the same time samples of milk were taken with the usual precautions, and later, these samples were investigated bacteriologically.

The blood-serum cultivations from the ulcers, in the case of both animals, showed a considerable number of colonies indistinguishable from those of the Klebs-Loeffler bacillus. These were subplated on serum and thus pure cultures were obtained.

The milk of both cows was centrifugalised and blood-serum tubes were inoculated from the deposit. In the cultures from both cases a few diphtheria-like bacilli could be demonstrated and by further plating pure cultures were obtained on serum.

In addition to the colonies of the diphtheria bacillus a number of colonies resembling them, but with a yellow tint, were observed and isolated. This bacillus, though morphologically resembling the *B. diphtheriae*, in twenty-four hours old cultures on solidified blood-serum was non-virulent for guinea-pigs, and further investigation revealed a number of characters differentiating it from the diphtheria bacillus. Numerous *Cocci* and other organisms were observed; *Streptococci* being especially numerous in the milk of the cow suffering from mammitis.

Brushings were made from the teats of thirteen apparently healthy cows at a small dairy farm. In two cases the cultures on solidified blood-serum showed diphtheria-like bacilli, but these on isolation were proved not to be the *B. diphtheriae*.

Bacteriological Examination of Brushing from Mrs F.'s throat.

This gave almost a pure culture of diphtheria-like bacilli which were similarly isolated in pure culture.

Examination of the Bacilli.

Morphologically the bacilli from the cows, from the milk, and from the patient's throat were found to be typical Klebs-Loeffler bacilli of medium size. The virulence of the three races was tested as shown in the following tables of experiments:—

Testing Bacillus from lesion in Cow 1.

A 24 hours' culture of the bacillus in alkaline broth was used :—

Guinea-pig No.	Weight of animal in g.	Dose of culture, etc. injected	Result
1	260	2 c.c. culture	Dead in 48 hrs.
2	278	4 c.c. culture	" " "
3	250	4 c.c. culture + 1 c.c. diph. anti-toxin	Lived. Very slight local reaction
4	255	5 c.c. diph. antitoxin and 24 hrs. later 2 c.c. culture	Lived. Very slight local reaction
5	268	5 c.c. diph. antitoxin and 24 hrs. later 4 c.c. culture	Lived. Very slight local reaction

The diphtheria antitoxin employed contained 300 units per c.c. (Ehrlich).

Testing Bacillus from milk of Cow 1. (A 24 hours' culture etc. as before.)

1	258	2 c.c. culture	Dead in 48 hrs.
2	258	4 c.c. culture	" " "
3	258	4 c.c. culture + 1 c.c. diph. anti-toxin	Lived. Very slight local reaction
4	248	5 c.c. diph. antitoxin and 24 hrs. later 2 c.c. culture	Lived. Very slight local reaction
5	250	5 c.c. diph. antitoxin and 24 hrs. later 4 c.c. culture	Lived. Very slight local reaction

Testing Bacillus from Mrs F.'s throat. (A 24 hours' culture etc. as before.)

1	255	2 c.c. culture	Dead in 48 hrs.
2	258	4 c.c. culture	" " "
3	250	4 c.c. culture + 1 c.c. diph. anti-toxin	Lived. Very slight local reaction
4	255	5 c.c. diph. antitoxin and 24 hrs. later 2 c.c. culture	Lived. Very slight local reaction
5	265	5 c.c. diph. antitoxin and 24 hrs. later 4 c.c. culture	Lived. Very slight local reaction

These experiments show that the three races of bacilli isolated from the cows, from the milk, and from the patient's throat, were virulent for guinea-pigs, and that diphtheria antitoxin given simultaneously or twenty-four hours before inoculation completely protected the experimental animals.

In addition to the tests mentioned, the object of which was to show that the organisms present were genuine *B. diphtherie*, a series of tests was carried out to ascertain the toxigenic power of the three races. As is well known the diphtheria bacillus from different sources varies greatly in this property, and without laying too great stress on the

matter we thought it might be an indication as to whether the three races in question were of common origin.

The three races, after being grown for two passages, on the usual alkaline broth employed in the preparation of diphtheria toxin, gave a good surface growth.

Twenty-seven test-tubes, each containing 10 c.c. of the same alkaline broth, were divided into three sets and each set was inoculated with one of the three races of bacilli. These test-tubes were placed in one incubator at 36° C. for ten days. The nine test-tubes of each series were then mixed and filtered through a Berkefeld filter. Our object in using a large number of test-tubes was to reduce the risk of accidental variations of toxicity. The toxins thus obtained were tested as follows:

Testing Toxin from Bacillus from lesion of Cow 1.

Guinea-pig No.	Weight in g.	Dose of toxin	Weight on successive days etc.				
			1	2	3	4	5
1	255	1.0 c.c.	245*	dead			
2	270	0.5	250*	dead			
3	262	0.2	246*	dead			
4	250	0.1	250*	225*	dead		
5	260	0.05	254*	245*	235*	225*	dead

Testing Toxin from Bacillus from Milk from Cow 1.

1	275	1.0	255*	dead			
2	258	0.5	250*	dead			
3	278	0.2	265*	dead			
4	270	0.1	255*	252*	245*	245*	dead
5	255	0.05	240*	235*	225*	225*	230

Testing Toxin from Bacillus from Mrs F.'s throat.

1	258	1.0 c.c.	dead				
2	265	0.5	dead				
3	250	0.2	dead				
4	265	0.1	235*	dead			
5	265	0.05	250*	240*	240*	230*	225*

Note: * = large local swelling.

The toxins from the three races, therefore, show a remarkable correspondence in toxicity.

The virulence of toxin-free cultures, obtained by growing the bacilli

24 hours in broth containing 1% glucose, was tested on guinea-pigs of 250 grammes weight with the following result:

Bacillus from Cow 1	killed at 0.2 c.c.
" "	milk of Cow 1	" 0.2 "
" "	throat of Mrs F.	" 0.1 "

A sample of blood taken from Cow 1 on January 23rd, and tested for diphtheria antitoxin, was found not to contain $\frac{1}{4}$ unit per c.c.

The Disease in the Cows.

With the view of further investigating the condition in the cow the two cows were removed on December 19th to an isolated cowhouse in the vicinity of the laboratory, and several miles from the place in which they had contracted the disease.

We obtained a young cow which had just had her first calf, which had been removed immediately after its birth. The cow was placed, on December 22nd, in a stall in the same shed in which the two affected cows were kept; but separated by a partition, and having a separate entrance. A new attendant, who had no connection with the outbreak and who had no other cattle in his charge, was instructed to milk the healthy cow after the affected animals. At first, from a misunderstanding, he carefully washed his hands after milking the diseased animals. On our attention being drawn to this the hand-washing between the milkings was stopped.

On the 31st December, the evening temperature of the new cow (Cow 3) rose to 103° F. and two days later there appeared on one of the teats a vesicle with clear contents, a representation of which is shown on Plate II, Figs. 2 and 3. A vesicle had evidently been already ruptured on the same teat. Cultivations from the fluid of the unruptured vesicle gave no diphtheria bacilli. Next day, at the base of the same teat, there could be felt and seen a papule the size of a pea. Two days later there were present on the teats and udder three lesions covered by crusts. Cultures from these again failed to demonstrate the presence of *B. diphtherie*.

Fresh crops of papules passing through the same stages occurred for about a fortnight, and a few of the crusts were still present nearly two months after the onset.

In this cow the process was not so severe as in the case of the first two animals in which the condition persisted somewhat longer.

This experiment demonstrates the contagious nature of the condition in the cow. In the fresh lesions, vesicles, and ulcers, we were unable to demonstrate the presence of the diphtheria bacillus. With the view of still further investigating the point as to whether this was a purely diphtheritic condition and not a diphtheria implanted on another process, we performed the following experiments on two calves:—

Calf 1. On January 6th the abdomen of a calf about six weeks old was shaved and scarified, and the scarification inoculated with crusts from the experimentally infected cow No. 3.

Calf 2. On the same day a second calf of about the same age which had forty-eight hours previously received subcutaneously 10,000 units of diphtheria antitoxin, was similarly shaved, scarified, and infected with the same material. Six days later the calves both showed a distinct eruption on the shaved area, consisting of about half-a-dozen flattened papules, almost petechial looking, with a diameter of about 3 mm., which later increased. This stage rapidly disappeared, giving place to small brown crusts, on removal of which there were seen shallow, smooth, moist ulcers, with slightly indurated edges. These scabs fell off in about a week and there was left a slight cicatrisation which could be felt about a month after the inoculation.

Although, as is shown in the figures from the two calves (see Plate II, Figs. 6 and 7), a slight difference was observable in the two cases, this was trivial, and was probably due to the difference in the texture and pigmentation of the skin. Cultivations were made from the crusts and subjacent areas in both cases on the sixth day after the onset of the eruption, but in neither case could diphtheria bacilli be demonstrated. These experiments appear to demonstrate:—

(1) That the calf was capable of being infected with the disease existing in the cow.

(2) That diphtheria antitoxin did not protect against this infection.

(3) That the diphtheria bacillus could not be found in the lesions in the calves.

These results obtained both from the cows and from the calves point to the conclusion that there was present a specific contagious eruptive condition apart from the diphtheritic infection.

Experiments on Vaccination.

As this condition in many of its features had a resemblance to natural vaccinia in the cow, we thought it desirable to carry out some experiments with the view of elucidating the point:—

Calves 1 and 2, which had already been successfully infected with the disease of the cow's udder, as previously described, were vaccinated on the buttock with Dr Chaumier's Lymph fourteen days later. In both cases a typical vaccinia resulted.

The same tube of vaccine lymph was used on several cases in the human subject, and gave rise to a severe but typical vaccinia. The inoculation of Cow 3 with vaccine lymph six weeks after the onset of the eruption gave rise to a typical vaccinia. Cows 1 and 2 were not vaccinated because as they were multiparae they might have already been the subjects of a naturally occurring vaccinia and have thus acquired a certain degree of immunity. The fact that inoculation with vaccine lymph gave rise to so typical a vaccination in the calves within a fortnight of infection with the material in question, and in the cow within six weeks of the naturally occurring infection, seems to almost preclude the possibility of vaccinia in the case of the original condition in the cows.

The converse experiment was performed on two calves, one of which was vaccinated with Chaumier's Lymph, the other kept as a control. An attempt was made about a fortnight later to infect these calves with the crusts from the cow's udder, but in both cases the experiment failed. This failure was no doubt due to the fact that, owing to a difficulty in obtaining calves, we were not able to carry out the experiment till a very late period in the condition of the cow, when the crusts were no longer capable of giving rise to the disease.

Crusts from the earlier stages of the disease in the cow, which had been preserved in 50% glycerine for six weeks, were also used, but these also failed both in the vaccinated and unvaccinated animals.

Summary.

In this outbreak of diphtheria certain individuals suffered from diphtheria, and others from sore throat probably diphtheritic. These individuals obtained their milk supply from two cows. Members of one household who did not drink milk, or who used it only after sterilisation, escaped infection. The cows yielding the milk were found to be

suffering from an eruptive disease of the udder, and both from the lesions and from the milk cultures of virulent diphtheria bacilli were isolated. The pathological condition in the cows preceded, by a short interval, the onset of the disease in the patients. Having regard to Power's epidemiological investigations, and to Klein's experimental work, this observation was of the greatest interest and naturally suggested the hypothesis that the lesions were due, primarily, to a specific diphtheritic infection of the cow. Further investigations weakened this view, for it must be noted:—

(1) That in a cow intentionally submitted to infection from the diseased cows and in which there occurred the eruptive condition of the udder, neither in the vesicular nor in the ulcerative stage of the disease could diphtheria bacilli be demonstrated.

(2) That in calves infected with the eruptive disease no diphtheria bacilli could be demonstrated.

(3) That in a calf, in spite of the fact that it had received 10,000 units of diphtheria antitoxin, the vesicular eruption was experimentally produced. The last is probably the strongest point in support of the dual nature of the condition in the cows.

It is conceivable that pathological lesions in the cow such as those described, if infected with the diphtheria bacillus, might form a suitable nidus for its growth and permit of the infection of large quantities of milk over a considerable period. Though we have as yet no evidence on the subject it is possible that a profound change in the virulence of the diphtheria bacillus for the human subject might be effected by such passage through the cow¹.

The disgusting habit the milkers in this part of England have, of spitting on their hands before milking, would easily account for the infection of the lesions, even in the absence of obvious diphtheria in the cowman; knowing, as we now do, that apparently healthy individuals are not uncommonly the hosts of the diphtheria bacillus.

The experiments made with the view of ascertaining whether the eruptive condition was genuine cowpox, are opposed to that view; for two calves, and one cow successfully infected with the eruptive condition and subsequently vaccinated with vaccine lymph, developed a typical vaccinia.

The somewhat limited time and material available have prevented

¹ A series of reports on a similar vesicular condition of the udder of the cow, investigated in relation to scarlatina, will be found in the Report of Prof. Brown, of the Agricultural Department of the Privy Council Office, in 1888.

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FIG. 1.

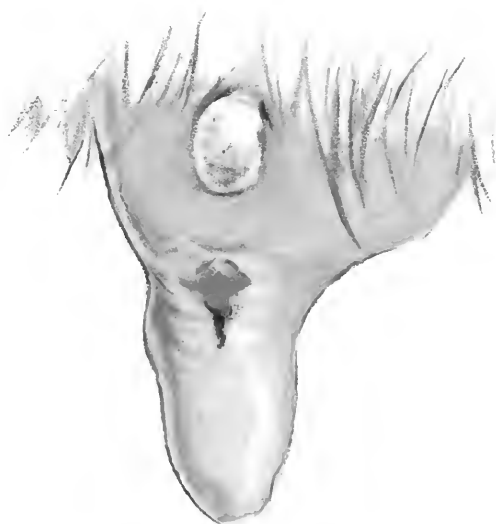


FIG. 2.



FIG. 3.



FIG. 4.



FIG. 5.



FIG. 6.



FIG. 7.

us from investigating the matter so fully as we should have wished, or on a scale proportionate to its interest and practical importance. We hope at some future time to go further into the matter.

Our best thanks are due to Mr Sidney Villar, F.R.C.V.S., for bringing the outbreak to our notice, and for his help in the veterinary side of the question; and to Dr Carson Smyth for his kindness in allowing us facilities for taking cultures from the throats of his patients.

DESCRIPTION OF PLATE.

PLATE II.

FIG. 1. Cow 1. Appearance of lesions when first observed on the udder.

FIGS. 2, 3, 4 and 5. Showing the condition in Cow 3 which had been intentionally exposed to infection.

FIGS. 2 and 3. Infected teat with intact and ruptured vesicles on the day of their appearance.

FIG. 4. Ditto 2 days later.

FIG. 5. Ditto 11 days later.

FIG. 6. Calf 1. Ten days after inoculation with material from Cow 3. This calf had received no antitoxin. Figure about one-half natural size.

FIG. 7. Calf 2. Ten days after inoculation with material from Cow 3. This calf had received 10000 units (Ehrlich) of Diphtheria antitoxin 48 hours before inoculation. Figure about one-half natural size.

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ON THE CONSTRUCTION AND USE OF LIFE-TABLES FROM A PUBLIC HEALTH POINT OF VIEW.

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THE following notes are to serve as a postscript to the paper which appeared under the above title in the preceding number of the *Journal of Hygiene* (pages 1—42).

I. *On a modification of the scheme of interpolation proposed on page 22.*

The following alternative scheme may be suggested as entailing less labour, and while not giving, on minute analysis, a p_x curve so perfect as the other, leading to ultimate l_x and E_x values not materially differing.

Series 1 has five orders of differences, and the remaining series 2, 3, 4, and 5, have each but four orders of differences.

Series					
1	$[p'_{.5}p'_{1.0}p'_{1.5}p'_{2.5}p'_{3.5}]p'_{4.5}$		
2	$p'_{1.5}[p'_{2.5}p'_{3.5}p'_{4.5}]p'_{5.5}$	
3	$p'_{2.5}[p'_{3.5}p'_{4.5}p'_{5.5}]p'_{6.5}$	
4	$p'_{3.5}[p'_{4.5}p'_{5.5}p'_{6.5}]p'_{7.5}$
5	$p'_{4.5}[p'_{5.5}p'_{6.5}p'_{7.5}p'_{8.5}...]$

The formulae on pages 22 and 24 are applicable to this scheme by simply eliminating respectively those relating to $\delta^8 u_0$ and δ^5 .

The checking equations for u_{40} and u_{30} are given on page 25.

However, since the paper above referred to has appeared in print an idea has occurred to the writer that it may be possible to dispense altogether with any scheme of analytical interpolation after the foundation series of p'_x values have been obtained, and to arrive at the required

series of p_x values for each separate year by a modification of the "graphic" method, thus combining in some degree the ease and simplicity of the latter with the accuracy of the former method. Accordingly what is next to be said may come under the following heading:

II. *On a suggested combination of the "analytical" and "graphic" methods in Life-Table construction.*

If it be assumed that the reader (1) has read and has at hand the paper already alluded to, and (2) is acquainted with the details of the graphic method, as explained in the last edition of Dr Newsholme's "Vital Statistics," the scheme now to be proposed may be made intelligible in very few words.

On reference to the preceding number of the *Journal* at pages 17—21 it will be evident that it is not a very difficult task to work out the foundation series of values of $\log p'_x$ given on page 21, especially as some simple rules will be given by which the actual calculation of $\log p'_5$, $\log p'_{10}$, and $\log p'_{15}$ may be dispensed with—at any rate they may or may not be calculated as preferred. The calculation of the values from $\log p'_{25}$ onwards is extremely simple and easy.

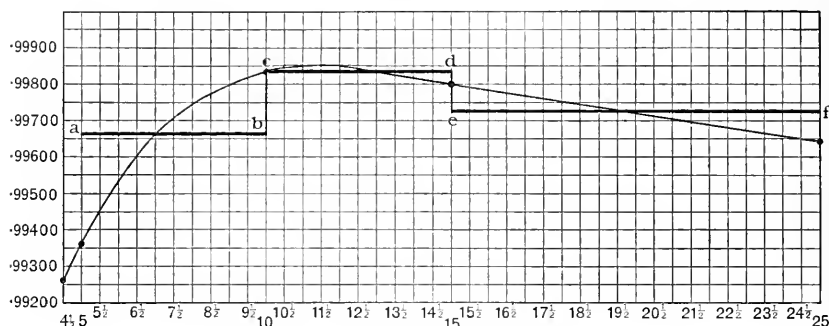
Having then obtained by an easy analytical method the series $\log p'_{25} \dots \log p'_{85}$, by differencing the series $\log p'_{45}$, $\log p'_{55}$, $\log p'_{65}$, $\log p'_{75}$ and $\log p'_{85}$ and carrying down the differences the values of $\log p'_{95}$ and of $\log p'_{105}$ are readily fixed.

It is also necessary to have the value of $\log p_4$ which will have been obtained at an earlier stage in the construction of the Life-Table, and as p_4 represents the chance of living from age 4 to age 5 it may be considered to be the chance of living a year at *exact age* $4\frac{1}{2}$, therefore $\log p_4$ may be called $\log p'_{4\frac{1}{2}}$.

The mode of procedure is to take a sheet or sheets of Layton's actuarial paper ruled into exact $\frac{1}{8}$ inch squares and mark out a base line on the scale of one division for a half-year; commencing with $4\frac{1}{2}$ (see diagram).

In order to construct the vertical scale the logs must be cut down to five figures and then each division may be made to represent '00050. The inconvenience of having to work on more than one sheet of paper, which would necessarily attend the use of Layton's paper, even on the comparatively small scale mentioned, may be obviated by obtaining some excellent paper made by Ch. Fortin & Cie., "no. 2 ruled into

$\frac{1}{8}$ inch squares." This may be obtained in lengths of several yards and it is wide enough to take in the whole curve from $4\frac{1}{2}$ to 95.



Assuming in the first instance that the values of $\log p'_5$, $\log p'_{10}$ and $\log p'_{15}$ have been calculated, it is now a simple matter to mark to scale the values of the logs in the ordinates corresponding to $4\frac{1}{2}$, 5, 10, 15, 25...105 and then a curved line may be drawn through the fixed points commencing with $4\frac{1}{2}$, the rule being simply to let the line be as little curved as possible consistently with running smoothly without angularities or breaks. It will be found that this curve is a much easier one to draw than those which have to be drawn to divide up population and deaths by the original graphic method.

The curve having been drawn it is simply necessary to measure the ordinates at $5\frac{1}{2}$, $6\frac{1}{2}$, $7\frac{1}{2}$, etc. etc., and these will give the required series of values of $\log p_5$, $\log p_6$, $\log p_7$, etc. etc. ready to be at once used for the next stage in the construction of the Life-Table.

However in order to avoid the necessity of calculating $\log p'_5$, $\log p'_{10}$ and $\log p'_{15}$, let the horizontal lines ab , cd , and ef be drawn as shown in the diagram, at heights above the base line corresponding to the values of the logs of the mean p_x values derived from the years of life and the total deaths in ten years for each of the age-periods respectively, 5—10, 10—15, and 15—25, by the fraction $\frac{2P-d}{2P+d}$. The formula being, (by logs), $\log p_x \text{ to } x+n = \log (2P-d) - \log (2P+d)$.

The position of $\log p'_{10}$ is fixed at the exact point c in the angle bcd , and the position of $\log p'_{15}$ at a point $\frac{3}{10}$ of the distance from d to e in the line de . It is not necessary to fix $\log p'_5$ at all, as its position will be indicated with sufficient accuracy by the point where the curve cuts the ordinate 5.

A curve of the shape shown in the diagram may now be drawn

through the fixed points, starting with the fixed point in the ordinate $4\frac{1}{2}$.

This may seem a very rough and ready method, but it has been deduced from actual calculation and plotting out in a number of instances.

For example in the particular instance which has been used for the diagram,

by calculation	by rule
$\log p'_{10} = \bar{1}.99836$	$\bar{1}.99839$
$\log p'_{15} = \bar{1}.99807$	$\bar{1}.99807$

As aids to accuracy in drawing this, the most difficult part of the curve, the following simple rules have been found to be applicable:

(1) The curve cuts the line ab on the ordinate 7, and the line cd on the ordinate 13.

(2) The highest point of the curve is on the ordinate $11\frac{1}{2}$.

(3) These rules apply to vertical and horizontal scales of similar relative proportions to those which have been recommended.

In the *Journal of the Royal Statistical Society*, Vol. LXII., Part iv. at pages 699—701, are to be found some remarks contributed by the writer by way of criticism of the graphic method as hitherto employed in Life-Table construction, the points alluded to being chiefly the extreme irregularity and want of symmetry of the p_x curves when plotted out from Life-Tables so constructed, and the impossible and absurd results obtained at the later ages of life.

By the use of the combined method herein suggested these difficulties would be obviated and results would be arrived at with less labour and probably greater accuracy than those obtained by the graphic division of population and deaths.

Although the present writer would personally prefer to use the previously described analytical method all throughout, for the sake of those who prefer to use a "graphic" method he can advance the following considerations to recommend the above-described scheme:

(1) The given *fixed points* are determined by a rigidly exact method.

(2) With a sufficient degree of technical skill in drawing and in measuring, a fairly close approximation can be obtained to the results which would be obtained by exact calculation—so close as to make but little difference in the ultimate E_x values.

(3) The differences from exact p_x values due to "personal equation" in drawing and in measuring, will certainly be less than the differences to be obtained by different systems of analytical interpolation.

By actual trial it has been found that the above described scheme gives very good results as far as age 65, but after this age there are difficulties in continuing the curve on the same scale. It would appear therefore preferable to construct the curve in two sections:—

(1) Let a curve be drawn as described as far as the ordinate 75. This is only to be used for measuring as far as $64\frac{1}{2}$.

(2) Let another curve be drawn from 55 to 95 with the vertical scale reduced to half, *i.e.* so that each division corresponds to $\cdot 00100$. This curve is to be used for measuring the ordinates from $65\frac{1}{2}$ to $94\frac{1}{2}$.

(3) By simply differencing the last five values obtained, *viz.* from $\log p' 90\frac{1}{2}$ to $\log p' 94\frac{1}{2}$, the series can be continued as far as may be required.

These or any other modifications of the proposed scheme must be left to the technical skill and to the discretion of those who may undertake to use it.

III. *Comparison of the results obtained by the simple rules which have been given for arriving at the values of $\log p'_{10}$ and $\log p'_{15}$ with the corresponding values worked out by exact calculation.*

Since the preceding pages have been in print the following Table has been worked out with the view of showing, by their application to an increased number of instances, to what extent the "rules" given, for arriving at the values of $\log p'_{10}$ and $\log p'_{15}$, may be relied upon for obtaining results approximating to those which would be got by calculation:

		(a) By calculation	(b) By rule	Differences of (b) from (a)
England and Wales 1881-90	Males	$\log p'_{10}$ $\bar{I}\cdot 99870$	$\bar{I}\cdot 99872$	+ $\cdot 00002$
		$\log p'_{15}$ $\bar{I}\cdot 99849$	$\bar{I}\cdot 99846$	- $\cdot 00003$
	Females	$\log p'_{10}$ $\bar{I}\cdot 99868$	$\bar{I}\cdot 99865$	- $\cdot 00003$
		$\log p'_{15}$ $\bar{I}\cdot 99840$	$\bar{I}\cdot 99841$	+ $\cdot 00001$
Selected Healthy Districts 1881-90	Males	$\log p'_{10}$ $\bar{I}\cdot 99897$	$\bar{I}\cdot 99901$	+ $\cdot 00004$
		$\log p'_{15}$ $\bar{I}\cdot 99886$	$\bar{I}\cdot 99877$	- $\cdot 00009$
	Females	$\log p'_{10}$ $\bar{I}\cdot 99885$	$\bar{I}\cdot 99882$	- $\cdot 00003$
		$\log p'_{15}$ $\bar{I}\cdot 99862$	$\bar{I}\cdot 99856$	- $\cdot 00006$
Manchester City 1881-90	Males	$\log p'_{10}$ $\bar{I}\cdot 99836$	$\bar{I}\cdot 99839$	+ $\cdot 00003$
		$\log p'_{15}$ $\bar{I}\cdot 99807$	$\bar{I}\cdot 99807$	$\pm \cdot 00000$
	Females	$\log p'_{10}$ $\bar{I}\cdot 99838$	$\bar{I}\cdot 99840$	+ $\cdot 00002$
		$\log p'_{15}$ $\bar{I}\cdot 99816$	$\bar{I}\cdot 99816$	$\pm \cdot 00000$
Brighton 1881-90	Males	$\log p'_{10}$ $\bar{I}\cdot 99900$	$\bar{I}\cdot 99900$	$\pm \cdot 00000$
		$\log p'_{15}$ $\bar{I}\cdot 99872$	$\bar{I}\cdot 99870$	- $\cdot 00002$
	Females	$\log p'_{10}$ $\bar{I}\cdot 99898$	$\bar{I}\cdot 99890$	- $\cdot 00008$
		$\log p'_{15}$ $\bar{I}\cdot 99877$	$\bar{I}\cdot 99882$	+ $\cdot 00005$
Glasgow 1881-90	Males	$\log p'_{10}$ $\bar{I}\cdot 99730$	$\bar{I}\cdot 99760$	+ $\cdot 00030$
		$\log p'_{15}$ $\bar{I}\cdot 99749$	$\bar{I}\cdot 99733$	- $\cdot 00016$
	Females	$\log p'_{10}$ $\bar{I}\cdot 99753$	$\bar{I}\cdot 99768$	+ $\cdot 00015$
		$\log p'_{15}$ $\bar{I}\cdot 99743$	$\bar{I}\cdot 99733$	- $\cdot 00010$

It is thus evident that, as regards the first four Life Tables, the results obtained by "rule" are very close to those arrived at by calculation, but that in the case of Glasgow, the results of the rules are rather more divergent from the true values than is desirable.

It would be, of course, more satisfactory *in every case* to take the trouble of calculating the values of $\log p'_5$, $\log p'_{10}$ and $\log p'_{15}$.

Some idea could be formed beforehand as to how closely the results of the rules would approximate to those to be calculated, by an inspection of the *death-rates* at the age-periods 5—10, 10—15, and 15—25. If these latter should be, as in the case of Glasgow, *very high*, the rules will probably produce less satisfactory results.

See the following *comparative Table of death-rates per thousand*.

Males.

Age-periods	England and Wales	Healthy Districts	Manchester City	Brighton	Glasgow
5—10	5.35	3.88	7.62	4.83	10.65
10—15	2.95	2.28	3.71	2.30	5.52
15—25	4.97	4.13	6.18	4.57	7.59

Females.

5—10	5.26	3.86	7.40	4.45	10.14
10—15	3.11	2.71	3.69	2.53	5.33
15—25	4.97	4.73	5.51	3.19	8.03

There is, however, no *direct* proportion between the magnitude of the death-rates and the accuracy of the results obtained by rule, for it will be noted that in the case of Manchester City, in which the death-rates come next below in order of magnitude, the results of the rules come out with extreme accuracy.

In the case of Glasgow the death-rates are not only very high but differ from those of Manchester in their relative proportions to each other.

IV. *Further suggestions for increasing the accuracy of the previously described "graphic" method.*

It is of course obvious that in drawing a curve through a number of fixed points, the shorter the intervals between these points the less is the possibility of variation in the curve when drawn by different individuals.

Thus, assuming that we have calculated the series of values $\log p'_5$, $\log p'_{10}$, $\log p'_{15}$... $\log p'_{85}$, while it is possible, by means of these fixed points alone, to draw a fairly satisfactory curve, it would be of great advantage, if it can be done without too much trouble, to fix intermediate points in each of the 10-yearly intervals.

The following simple formulae have been worked out so as to enable this to be done with comparatively little labour.

(1) *To obtain the value of $\log p'_{20}$, using the symbol u_x to represent $\log p'_x$,*

$$u_{20} = \frac{105u_5 - 576u_{10} + 1260u_{15} + 630u_{25} - 84u_{35} + 9u_{45}}{1344}.$$

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(2) To obtain the values of $\log p'_{30}$, $\log p'_{40}$, &c.

If there be six *equidistant* terms at an interval from each other of $2a$, then a central term, u_o , can be interpolated by the formula :

$$u_o = \frac{300(u_{-a} + u_a) - 50(u_{-3a} + u_{3a}) + 6(u_{-5a} + u_{5a})}{512}.$$

Thus to obtain $\log p'_{30}$, or u_{30} , since $a=5$,

$$u_{30} = \frac{300(u_{25} + u_{35}) - 50(u_{15} + u_{45}) + 6(u_5 + u_{55})}{512}.$$

In order to be able to obtain the values of u_{70} , u_{80} , u_{90} , and (if required) u_{100} , the series u_{45} , u_{55} , u_{65} , u_{75} , and u_{85} must be carried on by differencing so as to obtain the terms u_{95} , u_{105} , u_{115} , and u_{125} .

When this is done there will be a series of absolutely accurate fixed points, at intervals of 5 years, to start the curve with.

If desired by making $a=2\frac{1}{2}$, the same formula might be used with advantage, especially in the section of the curve after age 65, to fix the intermediate points $67\frac{1}{2}$, $72\frac{1}{2}$, $77\frac{1}{2}$, &c., &c.

The formula would be applicable to obtaining $u_{17\frac{1}{2}}$ to $u_{92\frac{1}{2}}$ inclusive, but in order to complete the series the following special formulae are needed :

$$\begin{aligned} u_{7\frac{1}{2}} &= u_{17\frac{1}{2}} - \left[\frac{(u_{20} - u_5) - 5(u_{10} - u_{15})}{4} \right], \\ u_{12\frac{1}{2}} &= \frac{5(u_{20} + 9u_{15} + 3u_{10}) - (u_5 + 24u_{17\frac{1}{2}})}{40}, \\ u_{97\frac{1}{2}} &= \frac{5(u_{90} + 9u_{95} + 3u_{100}) - (u_{105} + 24u_{92\frac{1}{2}})}{40}, \\ u_{102\frac{1}{2}} &= u_{92\frac{1}{2}} - \left[\frac{5(u_{95} - u_{100}) + (u_{90} - u_{105})}{4} \right]. \end{aligned}$$

As a point of practical convenience, in drawing the second section of the curve, from age 55 to age 95, it may be noted that it is desirable not only to reduce the vertical scale, but to increase the horizontal scale, so that $\frac{1}{8}$ inch will correspond to '00100 and $\frac{1}{4}$ year respectively.

With fixed points at shorter intervals than 10 years it is possible for each draughtsman according to his own convenience or preference to draw the curve in different sections and according to different scales.

To sum up it may be said that there are *three grades* in the application of the proposed graphic method :

(1) $\log p'_5$, $\log p'_{10}$, and $\log p'_{15}$ not calculated but determined by rule; $\log p'_{25}$,... $\log p'_{85}$ calculated; $\log p'_{95}$ found by differencing; curve drawn through these fixed points at 10-yearly intervals after age 15.

(2) $\log p'_5$, $\log p'_{10}$ and $\log p'_{15}$ calculated, and intermediate values $\log p'_{20}$ to $\log p'_{100}$ also fixed by calculation, so that the curve is drawn through fixed points at 5-yearly intervals.

(3) Intermediate values also fixed by calculation at intervals of $2\frac{1}{2}$ years. This, however, may be considered to involve too much labour for the purpose in view. Grade (2) will be found the most satisfactory.

The *practical use* of working out the series $\log p'_{7\frac{1}{2}}$, $\log p'_{12\frac{1}{2}}$, &c., &c., would be to compare the results obtained by grade (2) with those to be obtained by a complete analytical interpolation, without the trouble of going through the whole scheme as set down in Note I., and obtaining $\log p_x$ values for each separate year.

V. *Note on the calculation of $\log p'_{95}$ and $\log p'_{105}$.*

Instead of adopting the method already given, viz. by differencing the series $\log p'_{45} \dots \log p'_{85}$, an alternative procedure, theoretically better, would be to calculate $\log p'_{95}$ and $\log p'_{105}$ *directly* from population and deaths.

Thus, as the series of logs of $2P - d$ and $2P + d$ at age x and upwards, represented by the symbols $u_{45} \dots u_{85}$ (and $U_{45} \dots U_{85}$), will already have been carried down by differencing, to u_{95} and u_{105} , it will be a simple matter to carry on the series two stages further and thus obtain the terms u_{115} and u_{125} . When this has been done $\log p'_{95}$ and $\log p'_{105}$ can be worked out in exactly the same way as the series $\log p'_{25}$, &c.

$$\text{Thus} \quad \log p'_{95} = (u_{95} + \log b) - (U_{95} + \log B),$$

$$\text{and} \quad b = 8(u_{85} - u_{105}) - (u_{75} - u_{115}),$$

or, to use the most convenient form in working,

$$b = 10(u_{85} - u_{105}) - [2(u_{85} - u_{105}) + (u_{75} - u_{115})].$$

In order to calculate the hypothetical terms $\log p'_{115}$ and $\log p'_{125}$, the series $\log p'_{65} \dots \log p'_{105}$ can be differenced. It will be noted that all these values are derived from the series of population and deaths, $u_{45} \dots u_{85}$, and $U_{45} \dots U_{85}$.

In order to make the new analytical scheme of interpolation set down in Note I. at the beginning, on page 266, to correspond, two additional series, 7 and 8, commencing respectively with $\log p'_{55}$ and $\log p'_{65}$, would have to be appended, with "weldings" symmetrically arranged.

This method has been mentioned to show that it has been taken into consideration, and after having tried it in several instances the writer would still recommend the use of the simpler method already described.

VI. *An easy first lesson in constructing a Life-Table from the series of p_x values when obtained.*

For the sake of those who may find any difficulty in clearly comprehending the processes of calculation by which a Life-Table is built up on the foundation of the series of p_x values, the following simple Table may be of service.

Age	p_x	l_x	P_x	Q_x	$\frac{Q_x}{l_x} = E_x$
x	$\frac{8}{10} = 0.8$	10	9	25	$\frac{25}{10} = 2.5$
$x+1$	$\frac{8}{8} = 0.75$	8	7	16	$\frac{16}{8} = 2.0$
$x+2$	$\frac{6}{6} = 0.6$	6	5	9	$\frac{9}{6} = 1.5$
$x+3$	$\frac{4}{4} = 0.5$	4	3	4	$\frac{4}{4} = 1.0$
$x+4$	$\frac{2}{2} = 0$	2	1	1	$\frac{1}{2} = 0.5$
$x+5$		0			

The processes of calculation are as follows :

- (1) $l_x \times p_x = l_{x+1}$; $l_{x+1} \times p_{x+1} = l_{x+2}$; &c.
- (2) $P_x = \frac{1}{2} (l_x + l_{x+1})$; $P_{x+1} = \frac{1}{2} (l_{x+1} + l_{x+2})$; &c.
- (3) $Q_{x+4} = P_{x+4}$; $Q_{x+3} = Q_{x+4} + P_{x+3}$; &c.
- (4) $\frac{Q_x}{l_x} = E_x$; $\frac{Q_{x+1}}{l_{x+1}} = E_{x+1}$: &c.

It is of importance to comprehend :

- (1) that the p_x values have relation to the *present* tense ; i.e. each value depends upon the rate of mortality at the middle of the age x to $x+1$;
- (2) that the l_x values have relation to the *past* tense, in that they are only affected by the *preceding* p_x values, from p_0 to p_{x-1} ;
- (3) that the E_x values have relation to the *future* tense, in that they are only affected by p_x and the *following* values to $p_{x+\omega-1}$ (where $x+\omega$ is the age at which there are no more survivors).

It is obvious that the value $l_x = 10$ can only depend upon the arbitrary number l_0 with which the Life-Table will have been commenced, and upon the values of p_0 to p_{x-1} . Assuming these to have been so different that l_x has become 20 instead of 10, this could not affect the values of E_x to E_{x+4} , for the values of p_x to p_{x+4} remain the same, and therefore all the results of the calculations in the above Table will be doubled, and $E_x = \frac{50}{20} = 2.5$ as before.

Therefore Q_x is always in direct proportion to l_x .

A REVIEW OF CURRENT THEORIES REGARDING IMMUNITY.

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I.

THE primary object of these papers is to attempt to give a brief review of the present state of opinion regarding the many questions involved in the subject of immunity,—a review intended chiefly for those whose work is concerned with other fields of hygienic research. The carrying out of this aim has necessitated the re-statement of many facts familiar to bacteriologists, in order that the continuity of the discussion might be maintained. Wherever it has been considered advisable the details of the data which have formed the basis of theory have been given, but as a rule the leading results have been alone dealt with, partly because details might obscure the general principles it was intended to emphasise, partly because considerations of space have made an exhaustive treatment of the subject impossible.

For the present point of view we must neglect the microscopic structure of protoplasm, and think of living material as having even in its minutest presentations a molecular constitution of extraordinary chemical complexity. This complexity involves both the substances of which protoplasm is essentially composed and the materials which these substances elaborate in that metabolism, or capacity for activity, which we recognise as a very important manifestation of life. For metabolism, or the manufacture of new combinations, assimilation, or the taking up of extraneous material, is necessary. Now so far as any particular group of active living molecules is concerned, any such material with which these may come into contact, (1) may be capable of assimilation and may be used in metabolism, *i.e.* may be food, or (2) being capable of assimilation may disturb metabolism, *i.e.* may be poison, or (3) may be incapable

of assimilation, *i.e.* be what is usually called inert matter. In vital action there are also, of course, physical factors which either promote metabolism, interfere with it, or have no effect. It is with the second of the chemical interactions of protoplasm with external matter that we have especially to do, namely, with the process of poisoning. Next to the fact that such a process is possible, is the further fact that, while protoplasm may be seriously affected by a poison, it very frequently develops the capacity of tolerating the presence, and it may be the action, of a poison so that the metabolic activities ordinarily interfered with by the latter go on as usual. When this occurs the protoplasm is said to manifest immunity. This capacity is a vital factor in resistance to and recovery from disease.

Poisons are varied in their nature and mode of action, and in a complex colonial organism, such as an animal's body, an interference to a very small extent with the metabolism of a few cells may give rise to serious effects in the colony. But there appears to be a corresponding variety in the capacities of protoplasm to deal with interferences with its metabolism. Of the means by which tolerance to many kinds of poisons is established we know nothing, but with regard to tolerance against the pathogenic action of the bacteria we are beginning to have some understanding. The term immunity strictly applies to the development of tolerance towards any poison, but at present, on account of the fact that most work has been done on the processes by which tolerance arises against bacteria, the word is often limited to immunity against these bacteria, and it is in this sense that it is used in the present paper.

The two different types of bacterial action. There is little doubt that the pathogenic action of bacteria depends on a process of poisoning, but the results of enquiries regarding this process are very complicated. The complication arises from the fact that different bacteria interfere with metabolism in different ways, and in any discussion on immunity this cannot be too strongly insisted on. In the case of certain bacteria, such as those of diphtheria and tetanus, the organisms settle down in one part of the body and produce poisons, which, being absorbed, give rise to changes in the functions of relatively distant organs on which they have a selective action. Such bacteria, further, when grown in artificial media, produce poisons, which, after the actual bodies of the bacteria have been removed by filtration through porcelain, are capable of reproducing the characteristic symptoms of the disease. The actual nature of these poisons is unknown, for they have resisted all attempts at isolation in a pure

form. So far as any chemical reactions which they appear to possess go, they are to be grouped with a class of poisons fairly widely spread in nature, namely, with the snake poisons, the poisons of many other noxious creatures, and with certain vegetable poisons, the best known of which are ricin, derived from the castor-oil bean, and abrin, the active principle of the jequirity seed. In all these poisons the true nature is unknown, but they have this in common that they are precipitated by agents which precipitate those intermediate products of ordinary digestion,—the albumoses. This latter fact is, from our present point of view, not uninteresting and may be of importance, for it is possible that these poisons are in real constitution not far removed from the bodies which normally form the food of certain cells. Bacteria growing locally and producing effects by means of poisons or soluble toxins, as they are called, constitute then the *first* great group of such irritants. In the *second* group the disease effects are in some way or other more closely associated with the actual bodily presence of the bacteria themselves. The understanding of the action of the latter presents, however, many difficulties, for in many cases there is here also a certain tendency for the organisms to be local in their distribution. If we take the case of the pneumococcus we have a bacterium which is, in the usual manifestation of the disease it causes, confined to one organ of the body, and yet there is apparent evidence of effects on distant organs. Other instances of the same sort of action are found in the case of erysipelas and in the various forms of blood-poisoning, and, though to a much less extent, in typhoid fever. In man almost the only representatives of a true septicaemic process, *i.e.*, one where the organisms are found all over the body, are to be found in plague and in relapsing fever. In all the members of this second group of bacterial disorders, whether in their site the organisms are selective, as in the case of pneumonia or typhoid, or not, as in the case of the pyogenic cocci, the main general effect produced in the body has as its outstanding feature the development of fever, that condition whose true significance is not yet understood. Whether the latter is the cause of the other forms of disturbed metabolism which accompany it or whether all are part of one process has still to be ascertained. What we have to recognise in this connection is that the type of disordered metabolism is the same for all the diseases of this second group. Further, in all these diseases the local activity of the associated bacteria tends to be associated with the development of that complex of pathological changes summed up in the term inflammation. If we know little of the significance of the effects caused by such

bacteria, so we require more knowledge of the means by which these effects are produced. If cultures of the bacteria in question are filtered after the manner practised in the case of diphtheria and tetanus, the filtrates are often but little toxic; and even when they are there is sometimes evidence to be obtained that other and more powerful toxic agents have been left behind in the bacterial protoplasm. The proof of this is to be found in such facts as that observed by Metchnikoff, Roux, and Taurelli-Salimbeni ⁽¹⁾, namely, that an animal immunised against the filtered toxins of the cholera vibrio was not immune against an injection of the living organisms, and further that the serum of one animal immunised against the latter did not protect another animal against a fatal dose of the filtered toxin. Wassermann ⁽²⁾ found the same to be the case with the *Bacillus pyocyaneus*. These facts are difficult to understand. Whether the bacteria within the body produce toxins different from those produced in cultures, or whether the toxic effects produced in the body are due to the fact that bacteria die in the tissues in great numbers, that their bodies break up and liberate the poisons which they contain, and which do not under ordinary circumstances diffuse out of their protoplasm, or whether the changes in local metabolism produced by the inflammatory reaction which so often occurs are responsible for the changes in general metabolism, we do not know.

From the standpoint of immunity, in the case of both classes of bacterial disease the animal body requires to be protected both against the bacterial bodies and against their soluble toxins, but in the case of diseases of the first class protection is chiefly required against the latter, while in the second it is very likely that if the body can deal with the local effects of the bacterium this is what is chiefly necessary. In this latter statement is probably involved the fact that the neutralisation of the local effects usually means that the bacteria must be killed, but we cannot be dogmatic on this point. Certainly in very many, if not in all, cases the bacteria are killed. Here it may be remarked that it is probable, in certain cases, that the process of immunisation experimentally produced in animals may differ in some respects from the kind of immunity required in the disease naturally arising. Thus in cholera in man the disease is almost certainly a toxic one, for the bacteria are confined to the intestine. Animals are, however, not susceptible to infection by this path, though they do succumb to a disease process if the cholera vibrio is introduced into the peritoneal cavity. In the latter case if they are to be made artificially

to acquire immunity the important matter is that they should have immunity against the actual bacterium and not against its soluble poisons.

The methods of producing immunity against bacterial disease. While the general facts regarding immunity have been deduced from the observation of the consequences of recovery from disease arising under natural conditions, all our knowledge of what really takes place and all the important therapeutic results which have followed on this knowledge, have come from experimental enquiries conducted on animals. The process of the immunisation of these animals follows slightly different lines according to the group of bacterial noxae against which immunity is required. In the case of such bacteria as those of diphtheria and tetanus small doses of a weakened poison are first given, usually hypodermically, at intervals of a few days; these are succeeded by larger doses, and in a very short time the animal has acquired the capacity of withstanding, without symptoms, a dose of the virulent poison which in its former state would have inevitably killed it. It is now said to possess *active* immunity. But if the process of immunisation be carried further, then the serum of such an animal is found to possess *antitoxic* properties, *i.e.*, if injected into another animal in suitable amount it will prevent this second animal from contracting the disease if it be subsequently infected, and further if infection have already occurred in an animal the antitoxic serum has a therapeutic action. This transference of immunity is called, in the case of the second animal, the possession of *passive* immunity. Thus with regard to the diseases of the first group to which we have alluded, the fact that the chief action of the bacterium is effected by its soluble toxins, is reflected in the corresponding fact that, on the side of the animal, there are produced bodies which are capable of neutralising these toxins, and it may be further remarked that if the toxine and antitoxine be mixed *in vitro* in suitable proportions, and the mixture injected into an animal, nothing happens. In immunising animals against the second group of bacteria the same general procedures are adopted, except that here the actual bodies of the bacteria are injected,—killed cultures being often used in the initial stages. The same results so far as active and passive immunity are also obtained, but the therapeutic effects are not so good, for reasons which will appear later. Here the most important fact to be borne in mind is that the serum of the immune animal has *bactericidal* properties, and we have thus again the substance produced which is necessary for the neutralisation of the essential noxious agent. In the case of immun-

isation against the first group of bacteria, antitoxic sera are produced, in immunisation against the second group of bacteria, bactericidal sera are produced. To what these antitoxic and bactericidal sera owe their powers we cannot say, but in the case of the antitoxines the active material is probably a globulin (Brodie⁽³⁾, Nencki and Sieber⁽⁴⁾). Active and passive immunity as just described are to be grouped together as examples of *acquired* immunity, but it must also be borne in mind that there exists a *natural* immunity in the case of many species of animals against many diseases. This natural immunity is, however, usually not absolute and in most cases is not sufficient to protect the animal against every form of infection by the morbid agent.

We have now to proceed to enquire how the apparently comparatively simple facts detailed in the last paragraph are to be explained. These explanations lead us into discussions of the most complicated character and there are many points which are still obscure. As yet bacteriologists have been only able to deal with broad general principles,—in the case of no one disease have all the successive steps in the process been worked out. With regard to many of the principles involved the explanations at present rest largely on what occurs in circumstances analogous to those of bacterial infection, though there is little doubt that what appear now to be only analogies will be found to be examples of the same laws. There is a great probability that in different cases of immunity the details of the processes differ, though the general principles underlying all are the same. The differences in details are chiefly found to correspond with the two great classes of bacterial disease to which reference has been made. As we have already said probably in the development of immunity against every bacterium there requires to be developed a capacity of resistance to its toxins and also the capacity of actually killing the bacterium itself. As the former is the simpler process we shall first of all study it, and here it is usual to take as the typical diseases diphtheria and tetanus where the general toxic action overshadows altogether the local effects of the bacteria,—none the less, however, must it be remembered that such local effects do occur. We shall next take up the principles which underlie the capacity for killing bacteria in the animal body and especially the increase of these powers which accompanies the development of immunity. The diseases which have been mostly studied in this connection are cholera and typhoid and especially the artificial diseases caused by the bacteria when injected into animals. With regard to these diseases again it must be remembered that the animal body may require the

development of resistance to soluble toxines produced by the bacteria. It will be found that this part of the subject has had much light cast on it by the study of processes within the body analogous to the killing and dissolution of bacteria. The theories which have been advanced to account for the many observations regarding immunity have been varied; but in treating of them the task is somewhat lightened by the fact that at present all others are overshadowed by that associated with the name of Ehrlich, and round a discussion of this all that is essential in others can be taken up. This theory starts from certain researches on the nature of the soluble toxines, it then proceeds to treat of immunity against these toxines and therefore against the bacteria producing them, it then deals in similar fashion with the much more complicated question of immunity against infection by the members of our second group of bacteria. All forms of immunity, natural and acquired, and both the active and passive forms of the latter, are embraced by this theory, and we shall now proceed to enter into it in detail.

A. THE DEVELOPMENT OF THE CAPACITY OF RESISTING THE SOLUBLE TOXINES OF BACTERIA.

Ehrlich's views on the nature of soluble toxines. The first of the papers in which Ehrlich sets forth his views on immunity was published in 1897 and dealt with the constitution of the soluble toxines and the nature of antitoxine action. Since then his theories have been developed in a number of memoirs⁽⁵⁾. At that time he had formed the opinion that the union which, as we have seen, can take place *in vitro* between toxine and antitoxine is of a chemical nature. This was founded on such facts as that the two bodies can be titrated against one another like an acid and alkali, that union is hastened by warmth and is slower in the cold, that it takes place more readily with concentrated solutions of the substances than when these solutions are weak. The analogies with ordinary chemical reactions were thus very striking. On looking, however, more closely into the phenomena which accompany the neutralisation by the corresponding antitoxine of crude diphtheria toxine (*i.e.*, the fluid obtained by the filtration through unglazed porcelain of a bouillon growth of the bacillus) certain discrepancies appeared. The investigation of these led to the discovery of the fundamental facts on which the subsequent framework of research and deduction was based. To clear the ground we must observe that the strength of a toxine is measured by taking as a unit the amount which will kill a

guinea-pig weighing 250 grammes in four days. This is known as the minimal lethal dose ("M.L.D."). Theoretically the strength of an antitoxine is measured in terms of the so-called antitoxic unit, and the latter ought, as originally defined, to be the amount of the serum of an animal immunised against the disease under consideration which, when mixed with 100 M.L.D. of the toxine *in vitro* and allowed to stand for half-an-hour, will completely neutralise the poison, so that when the mixture is injected into a guinea-pig of the size just mentioned no symptoms will occur. In an extended series of observations Ehrlich first of all took one unit of antitoxine and found the amount of each of a number of samples of diphtheria toxine which this quantity exactly neutralised (*i.e.*, taking as the sign of neutralisation the fact that when the mixture was injected into a guinea-pig no symptoms occurred). He noticed that "of one toxine, perhaps 20, of a second, perhaps 50, and of yet a third, it might be 130 simple lethal doses were saturated by one immunity unit." From this statement it is evident that, whatever theoretical basis underlay the original standardisation of this antitoxine, the result was the setting up of a purely adventitious standard. The remark evidently applies to all strains of antitoxine in existence and may explain the unsatisfactory results obtained, especially formerly, in the therapeutic use of some of them. The next step showed a possible explanation of this anomaly. If in a mixture of toxine and antitoxine union took place in the way in which, say, a given amount of sodium hydrate in solution unites with the amount of hydrochloric acid calculated exactly to neutralise it, it is evident that the addition to a neutral mixture of toxine and antitoxine of one M.L.D. of toxine ought to cause the death of the test animal just as if one M.L.D. had been injected alone. This, however, was found not to be the case. Often many times the simple M.L.D. had to be added to the neutral mixture of one antitoxine unit with diphtheria toxine before, on injection, death was caused. In one sample of toxine investigated 28 M.L.D. had thus to be added to the neutral mixture before a fatal result was obtained, and the smallest amount observed was 1.7 M.L.D. For the other nine samples of toxine investigated, and which were either prepared by Ehrlich himself or obtained by him from other bacteriologists, the figures lay between the extremes given. To explain these results Ehrlich calls attention to the case of a toxine which immediately after filtration had an M.L.D. of .003 c.c. Nine months later the M.L.D. was .009 c.c., but it was found that, even after the lapse of this period, one antitoxine unit neutralised exactly the same quantity of the toxine as

at first. In other words one antitoxine unit when the toxine was freshly filtered neutralised 100·2 M.L.D., and nine months later only 33·4 M.L.D. It may be stated that the greatest care was taken to keep the antitoxine exactly in the same condition. In short, the toxic power of the toxine had decreased while its combining power had remained the same. That a toxine does diminish in strength on being kept had been a fact familiar to all workers, but this further fact that it still may require the same amount of antitoxine for neutralisation had not formerly been observed. For such degenerated toxines Ehrlich proposes the name "toxoids" to distinguish them from the true toxine which may be looked on as the most poisonous material present, and it is to be noted that probably every crude toxine, however fresh, contains both true toxine (henceforward referred to simply as "toxine") and toxoids, and Ehrlich considers that the fatal effect in four days, which is taken as the standard, is due to the toxine alone. He proceeds to give a theory to account for this phenomenon of loss of toxicity without loss of combining power. Suppose that in the ultimate toxine molecule there are two chemical affinities, such as occur in many bodies known to organic chemistry, and that the function of one,—called by Ehrlich the "haptophorous" group,—is to combine with the corresponding affinity in the antitoxine molecule, and that the function of the other,—the "toxophorous" group,—is to exert a poisonous action, then the difference between toxine and toxoid might be that in the latter these groups had undergone change. It is evident that with a loss of toxicity, such as we have seen occurs (caused on this theory by a degeneration of the toxophorous group), the haptophorous group might either be unaffected or it might also be degenerated; it is also theoretically possible that the change which inimically affected the toxophorous group might increase the potency of the haptophorous group. Take the case where both the haptophorous and toxophorous groups are degenerated, and consider the bearings of such a supposition on the fact that to a neutral mixture of crude toxine and antitoxine more than one M.L.D. has to be added to produce death in the test animal. In the neutral mixture there was both toxine and toxoid with the haptophorous groups of both satisfied. If, say, one M.L.D. of crude toxine is added this also contains toxine and toxoid, the amount of the former present being just sufficient to cause death. What will happen in the mixture will be that the toxine with its more powerful haptophorous groups will displace some of the toxoid already combined with the antitoxine, will combine with the latter and will thus be prevented from exercising its powerfully toxic

influence. The toxoids thus liberated, acting along with the toxoids in the M.L.D. added, may be insufficient to cause death, which in the case of diphtheria they can do, by causing a lingering illness with paralysis as a chief symptom. As a matter of fact, as we have seen reason to believe, a great many M.L.D. may have to be added before there is sufficient poisonous matter free to cause death in the prescribed time. Such, in outline sufficient for the purpose of being able to appreciate its bearing on the question of immunity, is the theory of Ehrlich regarding the constitution of the soluble toxines.

Method of action of soluble toxines and relation of action to production of antitoxines. Ehrlich next proceeds to develop from these views a theory of immunity against this class of poisons. The selective action of the morbid agent in diphtheria and tetanus was, of course, familiar to the clinician long before the true pathology of the diseases was known. Ehrlich accounts for this selective action as follows: As there is evidence of the existence of a chemical affinity between toxine and antitoxine, so, probably, there exists the same affinity between the toxine and corresponding affinities in the cells of the body, and the capacity of these affinities being mutually satisfied constitutes the susceptibility of the tissues. In short, the haptophorous group in the toxine fixes the latter in the cells and allows the toxophorous group to act, which it does by disturbing metabolic processes dependent on the activity of other molecules. Further, the production of antitoxine finds a possible explanation on this supposition. It is impossible to conceive that the affinities in the brain cells to which, say, the tetanus toxine becomes attached are ordinarily of no use in the cellular metabolism. In the latter they must bear a part or they would be examples of absolutely useless structures. Now the process of immunisation consists in its initial stages in the administration of small non-fatal doses of the pathogenic agent. Looking at the action of the first of these, though little or no toxic effect is produced, we see that the cells must be robbed of affinities, needed in ordinary metabolism, by the fact of the attachment to these affinities or "side-chains," or "receptors," as Ehrlich calls them¹, of toxine molecules. But it is a

¹ The term "side-chain" is unfortunate, as when applied to a chemical molecule whose constitution is known it has a definite meaning. Ehrlich has only used the term to express an analogy. It cannot but be wrong to speak, as is sometimes done, of the "side-chains" of a cell, though such side-chains may occur in molecules within the cell. The word "receptor" is much more fitting to express the group within the cells which may carry an affinity capable of saturation by a molecule outside the cell.

general biological law, on which the repair of many kinds of damage to the organism depends, that, if protoplasm be not too seriously injured, it tends to replace the damaged parts, and not only so, but, very frequently, it tends to over-regenerate the lost parts. Thus in the case under consideration, as has been specially pointed out by Weigert⁽⁶⁾, the affinities lost to the cell by having toxine anchored to them are reproduced; these new affinities are, however, again lost to the cell by saturation by the further doses of toxine injected in the immunisation process, and here it is to be specially borne in mind that there is always a progressive increase of the amounts of toxines injected. The cell thus has to go on manufacturing the affinities whose use it is constantly losing, and finally the latter are formed in such enormous numbers as to be present in proportions altogether beyond the cellular requirements. They are, therefore, being no longer of any use to the cell, waste material and are excreted accordingly. They thus pass into the serum and form the antitoxic agent in the latter, for they retain the original capacity they possessed within the cell of combining with the toxine molecules. When these cast-off receptors or "side-chains" of the susceptible cell meet the toxine their free affinities saturate the haptophorous group of the latter, which thus loses the means by which it becomes attached to cells, and therefore, as anchoring is impossible, the toxophorous group of the toxine no longer has the opportunity of working a pathogenic action. The toxine is, in fact, bereft of its toxic power. If the toxine is saturated thus with antitoxine and injected into an animal's body nothing occurs,—if the saturation takes place within an animal's body the mixture is again inert, and its fate in either case we do not know.

Discussion of Ehrlich's theory. Such are Ehrlich's views on the origin of immunity against bacteria giving rise to soluble poisons and on the nature of the interactions of toxines and antitoxines, and it must at once be admitted that they have opened up entirely new ground. Up to the time of their publication bacteriologists had been aiming at the obtaining by chemical means of pure samples of the substances involved, in order that their properties might be studied. For this method Ehrlich substituted one of analysis by the study of the physiological results. We therefore pass to enquire how this study and the theories based upon it stand in relation to other lines of research on the questions at issue. We shall first here look at the evidence for Ehrlich's fundamental canon that the union between toxine and antitoxine is a chemical one, secondly, at what

evidence there is for his views on the constitution of the toxins, thirdly, at the evidence from other sources of his views on the origin and development of the antitoxines, and, finally, discuss certain difficulties which the adoption of the view may seem to raise.

(1) *The nature of the antagonism between toxine and antitoxine.* Two theories as to the interaction of these substances have been put forward, one based on chemical, the other on physiological grounds. According to the former view (that adopted by Ehrlich) antitoxine neutralises toxine in the way that an alkali neutralises an acid. According to the latter, there is no such interaction, but, when both are present in the body of an animal, the antitoxine stimulates the tissues to resist the toxine. The evidence for the physiological view rests chiefly on three experiments. Buchner⁽⁷⁾ is stated to have taken a mixture of tetanus toxine and antitoxine which was quite neutral to the mouse, and to have found that it was not neutral to the guinea-pig. From this he concluded that no actual combination of the two substances had taken place. His view was that as, weight for weight, the guinea-pig is a more susceptible animal towards tetanus than the mouse, neutrality for the latter meant that there was present in the mixture named enough of the antitoxine to enable the animal to resist the toxine, while for the guinea-pig more stimulation would be required and enough antitoxine was not present. Calmette⁽⁸⁾ adduced another series of experiments to support a similar view. The antitoxine to certain serpent poisons is more susceptible to heat than the actual poison, the opposite being the case with tetanus and diphtheria toxins. This investigator took a mixture of venene and antivenene which he stated was neutral and heated it, and he found that its toxicity was restored, from which he deduced that no interaction *in vitro* had taken place, but that the two bodies simply existed side by side unchanged. Wassermann⁽⁹⁾ obtained a similar result with the antitoxine of the soluble poison of the *Bacillus pyocyaneus*. The former substance is destroyed by boiling, while the toxine is not. A mixture of the two bodies said to be neutral regains its toxicity when heated, and further the toxicity was again lost if fresh antitoxine was added.

Let us look more closely at these results. If Buchner's experiments be carefully studied it will be found that undoubtedly he was dealing with mixtures which were not neutral even for mice. He first took 10 mice and found that .0001 gramme of a particular toxine was an unfailing fatal dose. Whether it was the M.L.D. in the modern sense of the phrase cannot be determined. In the case of a comparative

experiment on 10 guinea-pigs (whose weight was on an average 20 times that of the mice) the same dose was not fatal, though it gave rise to slight tetanic symptoms. In the test experiment 23 mice received each '014 gramme of toxine (*i.e.* 140 times the fatal dose) mixed with '00135 gramme of antitoxine, an amount which was said to be sufficient to neutralise the 140 fatal doses. It is evident that the basis on which the latter amount of antitoxine was calculated was the amount of antitoxine supposed to be capable of neutralising '0001 gramme of toxine, for in the case of three of the mice death from tetanus occurred, and 11 suffered from slight tetanus. Only in nine cases were no symptoms of the disease observed. The mixture of toxine and antitoxine was, it is thus evident, not neutral for the majority of mice. In the case of 23 guinea-pigs injected with the same mixtures 8 died, 12 suffered from tetanus, and 3 had no symptoms. Thus the greater susceptibility of the latter species of animal was only evidenced by its succumbing more easily to a dose of non-neutralised toxine. Calmette's results have been criticised by C. J. Martin and Cherry⁽¹⁰⁾, who have shown that in them the importance of time as a factor in complete neutralisation had been neglected. If the apparently neutral mixture were allowed to stand long enough *in vitro*, at the end of a given period no return of toxicity on heating could be obtained. Apparently the mixtures used by Calmette were not really neutral, but there was sufficient excess of free antitoxine to prevent the toxine, which at the moment of injection was still unneutralised, from having a toxic effect on the animals. Union in fact here was completed within the animal's body. So far as appears, a similar test has not been applied in the case of Wassermann's results, but this would have to be done before they were finally accepted as evidence in favour of a physiological explanation of the reaction of toxine and antitoxine. The general conclusion to be drawn is that, at present, there is no evidence of the action of antitoxine being a physiological one to which cogent objection cannot be urged.

In favour of the view that the reaction between toxine and antitoxine is really of a chemical nature, several facts can be adduced. That the reaction takes place more readily when the respective solutions are concentrated and when they are warmed has been confirmed by Knorr⁽¹¹⁾. Again, C. J. Martin and Cherry have adduced other evidence pointing to the same conclusion. They have investigated the behaviour of various albuminous substances when these are filtered under high pres-

sure through a porcelain filter, the pores of which have been filled with gelatine. A very strongly supported dialyser is thus, to all intents and purposes, formed. They found that through such a filter antitoxine did not pass, while toxine did. Mixtures of toxine and antitoxine were allowed to stand for varying times and then subjected to filtration. The longer the mixture was allowed to stand before being filtered the less toxine passed through, till a time arrived when no toxine appeared in the filtrate and further what had not passed through the filter was found to be non-toxic. This indicates that a combination between the two bodies took place when they were left long enough in contact. Ehrlich ⁽¹²⁾ has brought forward other results besides those mentioned which support his view. A poison, ricin, can be extracted from the castor-oil bean, and among its other properties is the capacity of dissolving red blood corpuscles. Animals can be immunised against its poisonous action by a process identical with that employed in immunisation against tetanus or diphtheria. The serum of these animals contains an antiricin corresponding to an antitoxine. By experiments in test-tubes it can be shown that the blood corpuscles of, say, the rabbit can be protected against the action of the ricin by means of this anti-ricin. Now red blood corpuscles are usually looked on as mere carriers of oxygen and incapable of physiological reaction such as is presupposed to take place in a cell when it is stimulated to resist an external noxious agent. Such a response to a stimulus would have to be supposed to take place in the blood corpuscle, *i.e.* a physiological view of the antagonism of the two substances would have to be taken, if the action of anti-ricin on the ricin were not merely a chemical one. Again, strong support of the chemical view is obtained from a more than analogous case of another anti-body. As is well known, rennet curdles milk. Morgenroth ⁽¹³⁾ injected after the manner of an immunisation this ferment into a goat, and he found that the serum of the animal acquired a property of protecting milk against the action of the ferment. No one will deny that milk is an inert substance quite incapable of physiological reaction, and therefore there is little doubt that the reaction of the antirennet on the rennet is of a chemical nature. Taking into consideration all the facts bearing on the interaction of toxins and analogous bodies on the corresponding toxins, we must hold that, in the absence of direct experiment with pure samples of the substances in question, there is very strong presumptive evidence that these substances combine after the manner of ordinary chemical bodies which have affinities for one another.

(2) *The confirmation of Ehrlich's views as to the constitution of toxines.* We now pass to enquire if confirmation is forthcoming of Ehrlich's views of the degeneration of toxines and of the existence in the toxine molecule of independent binding and poisonous groups. The most weighty contribution here is the work of Madsen⁽¹⁴⁾ on the poison known as tetanolysin. Ehrlich⁽¹⁵⁾ first noticed that in certain bouillon cultures of the tetanus bacillus, though not in all, there occurred the property of dissolving the red blood corpuscles of certain animals. That this property is probably dependent on a poison distinct from that which gives rise to the spasms of tetanus, is indicated by the fact that it is not possessed by all bouillon cultures, and further that it is very readily lost, as, for example, by heating for 20 minutes to 50° C.—a temperature which will scarcely affect the spasm-producing action. Ehrlich therefore calls the substance tetano-lysin, and the ordinary spasm-producing body tetano-spasmin. With regard to this tetanolysin when an animal is immunised by a bouillon containing it, its serum contains an anti-body which will protect the susceptible blood corpuscles against the dissolving action. Taking advantage of these facts Madsen has investigated the constitution of this poison along the same lines as those pursued by Ehrlich with the diphtheria toxine. The only difference in the method was that the effects of different mixtures of the toxine and antitoxine on the blood corpuscles were observed in test-tubes, instead of as in Ehrlich's experiments by injecting the mixtures into guinea-pigs. In this way not only could many more experiments be done at one time, but the possibility of a physiological action of the antitetano-spasmin was excluded. The results were to show that the crude tetanolysin of the bouillon culture was not a single substance, but contained, besides the most potent body, another which, while requiring the same amount of antitoxine for its neutralisation, had much less haemolytic action. Further the crude substance contained also other bodies which had less combining power associated with less haemolytic action. In fact, the investigation of this substance, under circumstances where only chemical reactions could take place, showed it to have an absolutely analogous constitution to that which Ehrlich had assigned to the diphtheria toxine. It may here be added that Ehrlich's experiments with diphtheria poison have been confirmed by Bulloch⁽¹⁶⁾. There is thus the strongest ground for believing that in such crude toxines as that of diphtheria there is a mixture of bodies. All of these possess two unsaturated affinities, one associated with the capacity of combining with antitoxine, the other

having a toxic action, and the differences between the different bodies present in the crude toxine are differences chiefly in the toxophorous group, though differences also occur in the haptophorous group. It may be said that Ehrlich has devised experiments by which the relative amount of toxoid and toxine in the crude toxine can be estimated. It would lead us too far afield to go into this matter fully, though the results confirm generally the soundness of his physiological analyses of toxines. Roughly speaking the method consists in first determining the amount of, say, crude diphtheria toxine which will exactly be neutralised by one immunity unit of antitoxine. In a long series of animals the effects are now studied of adding in each case one two-hundredth less of antitoxine to such a dose of toxine and injecting the mixture. Now in one such series the animals up to that which received the toxine *plus* the one-hundred-and-sixty-seven two-hundredths of one antitoxine unit died of paralysis after long illnesses, while in animals which received less than this amount of antitoxine death with acute symptoms occurred in a few days. In other words, up to the point named there was enough antitoxine still present to completely neutralise all the toxine (with its stronger haptophorous group), and the symptoms were produced by the toxoids. Below the point named there was toxine unsaturated and thus rapid death was produced. In such a crude toxine, according to Ehrlich's nomenclature, there would be reckoned to be 33 toxoid equivalents. This experiment is only cited to bring forward another point, namely, the question as to the nature of the relation of the toxines to the toxoids. Dreyer and Madsen⁽¹⁷⁾ (one of whose experiments furnished the figures just quoted) have immunised animals by means of mixtures of toxines and toxoids in which the toxine part was completely saturated by antitoxine. They found that the antitoxine present in the sera of these animals neutralised ordinary crude diphtheria toxine, *i.e.* containing both toxine and toxoid. Therefore there is strong ground for supposing that the haptophorous group in toxoid is the same as that in toxine. This is fresh support to Ehrlich's views.

(3) *The evidence in support of Ehrlich's views on the origin and development of immunity.* This part of the subject divides itself into two parts, firstly, the evidence for the fixation of the toxine in the bodily cells, and, secondly, the evidence for the production and over-regeneration of the antitoxine by these cells. We may first of all here clear the way by qualifying a quasi-popular statement as regards the development of immunity in an animal. It is usually said, and the statement is often quite true, that an attack of a disease, which

has been recovered from, protects the individual from fresh infection. In the study of immunisation experiments, however, it is found that the development of disease symptoms is not necessary to the production of immunity. In immunising animals for the purpose of obtaining from their blood an antitoxine for use as a therapeutic agent, it has long been the custom to commence the process by using a toxine whose toxicity has been impaired by the action of such agents as iodine or terchloride of iodine. In such cases often no symptoms of disease may manifest themselves. The present writer⁽¹⁸⁾ has studied a curious reaction of tetanus toxine bearing on this matter. If the toxine be acted on by hydrochloric acid it gradually loses its toxicity, but, after all toxicity has apparently gone, a certain degree can be made to return if the acid be neutralised by sodium hydrate. It was found, however, that, during this period when the toxicity was only held in check, repeated doses of the acid mixture produced definite immunity in guinea-pigs, no tetanic symptoms being caused. If we accept Ehrlich's view of the constitution of such a toxine we would say that here the toxoporous group had been destroyed while the haptoporous group had still the capacity of combining with susceptible cells and producing immunity. Whether we use this phraseology or not, we must admit that a toxine can lose its toxicity without losing its capacity of producing immunity, and therefore immunity can be produced without an animal suffering from the disease. This is a not unimportant point in support of Ehrlich's theory.

We now come to examine *the evidence for the fixation of such toxins as those of diphtheria and tetanus in the bodies of the animals in whom immunity is capable of being produced*. Here we may first look at certain experiments by Dönitz⁽¹⁹⁾. This observer determined the amount of tetanus antitoxine which would neutralise 12 M.L.D. of a particular toxine. He then injected the latter amount into the vein of one ear of each of a series of rabbits, and into the vein of the other ear he injected quantities of the antitoxine at intervals after the toxine injection which varied with different animals. He found that while, if the mixture were made before injection the amount of toxine mentioned was neutralised by 1 c.c. of a 1 in 2000 dilution of the antitoxine, if injection of antitoxine took place 4 minutes after the toxine injection 1 c.c. of a 1 in 600 dilution was necessary—otherwise death occurred—if 8 min., 1 c.c. of a 1 in 200 dilution, if 15 min. 1 c.c. of a 1 in 100 solution. He found that similar facts were true of the amount of diphtheria antitoxine required to neutralise diphtheria toxine. From the facts regarding tetanus he concludes that at least

within 8 min. of the toxine being injected enough is fixed in the animal's body to cause death. Heymans⁽²⁰⁾ found that, if all the blood were removed from an animal a few minutes after the injection of a M.L.D. of tetanus toxine and the blood of another animal substituted, still the animal died of tetanus. This is still more conclusive evidence in the same direction.

When we come to enquire where the toxine is fixed we are face to face with a very difficult question. It is natural that in attempts at its solution attention should have been largely directed to what takes place in tetanus, for, as has already been remarked, in this disease there is strong clinical evidence of a selective action on the part of the poison for the central nervous system. The experiments usually brought forward here are those of Wassermann and Takaki⁽²¹⁾. These observers took the brain of the guinea-pig, an animal very susceptible to tetanus, and bruising it thoroughly in a mortar mixed it with varying amounts of tetanus toxine. They found that it was capable of neutralising a considerable amount of the poison. Not only so but if an emulsion of the brain were injected within the 24 hours previous to the injection of toxine the latter appeared to be neutralised. This property of the brain was not shared by the other organs of the body. The deduction drawn from these experiments was that the brain substance acted on the toxine in the way that antitoxine does, and they are accepted by Ehrlich as bearing out his view as to the source of the latter substance. This work has given rise to a great deal of controversy, and the view of the authors and of Ehrlich has been combated by many observers. That the neutralisation takes place has been confirmed by Knorr⁽²²⁾, Metchnikoff⁽²³⁾, Roux and Borrel⁽²⁴⁾, Danysz⁽²⁵⁾ and Marie⁽²⁶⁾. Several objections are, however, raised by these observers. Metchnikoff has been unable to find that the brain possesses any more antitetanic power than the other organs of the body. Danysz has found that if the apparently neutral mixture of brain and toxine be subjected to maceration with 75 per cent. sodium chloride a certain amount of the toxine again passes into solution—a fact unlike anything which happens in mixtures of toxine and ordinary antitoxine. He has further made the very important observation that if the brain be heated to 100° C. it still retains its neutralising properties. Now, according to the results of Tizzoni and Cattani⁽²⁷⁾, tetanus antitoxine is destroyed at 68° C., *i.e.* loses its power of neutralising toxine. It would therefore appear as if the body, which, in the emulsion of brain used, neutralises toxine, may differ from true antitoxine. The view

of the four observers last named, is that the neutralisation is not of the nature of a chemical union but that it is a mere entanglement of the toxine by the *débris* of the nerve cells. The reason given for its not giving rise to tetanus when the mixture containing it is injected into an animal is that the cellular *débris* is taken up by leucocytes and that within the latter the toxine is destroyed. This explanation is rather difficult to accept. If there is free toxine present in the mixture then according to the results of Vaillard and Rouget⁽²⁸⁾ the leucocytes will be repelled, for this is the effect produced by the poison in question—an effect which according to the observers just mentioned is the explanation of the fact that tetanus spores deprived by heat of the small amount of toxine naturally adhering to them are taken up by phagocytes and prevented from causing death. If toxine be present they are not thus taken up and thus tetanus follows. Shortly after the publication of the first paper by Wassermann and Takaki it was stated by Ransom⁽²⁹⁾ that the brain of the fowl, which is very insusceptible to tetanus, did not fix the toxine. This observation has not been confirmed, for Knorr (*loc. cit.*) found that there was very little difference in the fixative properties of this animal's brain and that of the guinea-pig.

This question of the fixation of toxine has been by many observers confused by the introduction of side issues. Thus Metchnikoff adduces as evidence of the non-fixation of toxine by the central nervous system of the fowl the fact that in an animal treated with tetanus toxine the other organs of the body may be more antitetanic than the brain and spinal cord. He has found the same to be true of the alligator, which is non-susceptible to tetanus but which will develop a fairly strong anti-tetanic serum if treated with tetanus toxine. Such an occurrence does not in the least detract from Ehrlich's theory. There is evidence that in this disease other organs besides the brain can fix the toxine. This is seen from certain experiments of Roux and Borrel (*loc. cit.*). In these it was found that in the rabbit, while one-tenth of a c.c. of toxine produced tetanus of a fatal kind when introduced into the brain, 2.5 c.c. was the fatal dose if introduced under the skin. In the guinea-pig on the contrary one-hundredth of a c.c. introduced subcutaneously caused death in 50 hours, while the same amount given intra-cerebrally caused death in three days. Such results are distinctly in favour of Ehrlich's view, for in the case of the rabbit the extra amount of toxine must have been taken up by tissues other than the nervous system. In the guinea-pig, on the other hand, all the toxine must have gone directly

to the latter. Dönitz (*loc. cit.*) had previously suggested that something of this kind can occur in the rabbit to explain what he calls *tetanus sine tetano*. In this phrase he refers to the fact that when nearly neutral doses of mixtures of toxine and antitoxine are given to this animal sometimes death does not occur from tetanus but from a kind of cachexia. It is quite conceivable that toxine can be fixed by cells, interference with whose function is not of such vital importance to the body as the nerve cells, and that it is only under certain very special circumstances that the pathogenic effects of such cells being affected manifest themselves. It is to be remarked, however, in this connection that from Ehrlich's standpoint all these sites where the toxine is fixed are potential sites of antitoxine formation, and, therefore, in such a disease as tetanus, antitoxine may be formed in a variety of organs. It is possible, again accepting Ehrlich's position, that in such an animal as the alligator the explanation of its insusceptibility to tetanus along with its capacity of forming antitoxine may be that the nervous system cannot fix the toxine while other organs can, and it is in these that antitoxine is produced.

It must be admitted that the evidence with regard to the fixation of toxines is very unsatisfactory, and much further investigation is here necessary before Ehrlich's position can be unreservedly accepted. It is to be remarked, however, that the methods which have hitherto been applied to the solution of this question have been of a somewhat insufficient kind, for the work of Buchner⁽²⁰⁾ on the sugar-fermenting substance in yeast has shown that the mere bruising of cells in an ordinary way is probably a very uncertain method of obtaining the intracellular juices.

It is evident that according to Ehrlich's theory *the question of the site of fixation of toxine is co-related to that of the site of antitoxine formation*, for if the theory be correct where the toxine is fixed there the antitoxine is formed. Not only are the attempts to determine the place of toxine fixation rather unsatisfactory, but what has been found regarding the relative richness in antitoxine of the different organs of the bodies of immunised animals does not shed any light on the question of the site of formation of this substance. Metchnikoff, in a guinea-pig immune to tetanus, found that all the organs, the brain included, had less antitoxic power than the blood, the kidney being the only organ that had any great antitoxic value. These results are borne out by Dzierzowski⁽²¹⁾, who, in a horse immune to diphtheria, found the kidneys and supra-renals more rich in antitoxine than the other

organs. The chief conclusion the latter observer draws from his work is that the antitoxine is excreted by the urine and, also, it may be incidentally mentioned, by the sweat.

The general conclusion to be drawn as to this branch of the subject is that, while such experiments as those of Dönitz and Heymans leave little doubt that such a toxine as that of tetanus is rapidly taken from the blood into the organs, more research is necessary as to the site of its fixation. More knowledge is also required as to the site of antitoxine formation.

While the site of antitoxine formation in the body is obscure, certain facts are known regarding what happens in the course of its development which require attention. From time to time it has been suggested that antitoxine might really be a modified toxine. This view was first put forward by Buchner⁽³²⁾ on theoretical grounds. Such an idea has some fascination, or rather had, because up till the time of Ehrlich's theory no definite attempt had been made to co-relate the occurrences of the process of immunity with any normal function of cells. It thus seemed very unintelligible that an animal, which had never been subjected under natural conditions to infection by a toxic agent such as ricin poison, should all the same be at once able not only to develop immunity to it, but should be able to transfer a certain degree of this immunity by means of its serum to another individual. It might thus appear natural to think that the substance, by which this immunity was transferred, was only a modification of the toxic agent. No facts, however, can be advanced to support this view, for the great difficulty here has been that, as we shall see presently, the amount of antitoxine developed in an animal's body is very much greater than the amount of toxine which was used to produce the immunity. To get over this difficulty it has been suggested that the toxine molecule might split up into a series of molecules of less size, each of which might contain a group capable of neutralising a toxine molecule. On theoretical grounds the existence of such molecules is not impossible, but unfortunately all the evidence we have (Brodie, Martin and Cherry, *loc. cit.*) goes to show that the antitoxine molecules are larger than those of the toxins. The following considerations support the view that antitoxine is formed somewhere in the body and during the course of its formation is shed out into the blood. Roux and Vaillard⁽³³⁾ took a rabbit immune against tetanus and possessing a strongly antitetanic serum and in the course of a few days removed from it a quantity of blood equal to the total amount of blood calculated originally to be in its body. This was

not followed by a sensible diminution in the antitoxic value of the serum. Similar and more exact experiments by Salamonsen and Madsen⁽³⁴⁾ on goats immunised against diphtheria had a similar result. These observers removed large proportions of the animals' blood and at once substituted for it normal saline solution. They noticed that immediately after the bleeding there was a fall in the antitoxic value of the serum, which corresponded in degree to the dilution of the blood by the saline injected. But after a short period of time there was again a rise in the antitoxic value. Both of these sets of experiments show that, when antitoxine is removed from an animal's body by removing its blood, there is after a time a fresh passage of the antitoxic substance into the circulating fluids.

The next point to be considered is the relation of the amount of antitoxine produced to the amount of toxine injected in the immunisation process. Knorr (*loc. cit.*) showed in one experiment that, in a horse already furnishing an antitoxic serum, the injection of as much toxine as could be neutralised by one unit of antitoxine was followed by the production of 100,000 times that amount of antitoxine. This is no doubt an extreme case but it illustrates the capacities of immunisation. I have obtained (*loc. cit.*) similar results in another way. If tetanus toxine be acted on by hydrochloric acid until its toxicity is destroyed, it still retains the capacity of giving rise to immunity. By acting for the same time on different moieties of toxine there is no doubt that on each occasion the state of the modified toxine would be the same. Guinea-pigs were immunised by this modified toxine and instead of gradually increasing doses being used the amount was kept the same. One set of animals received four doses of the modified toxine and another set received eight such doses. Of the serum of the first .5 gramme was required to protect a guinea-pig against an M.L.D., while of the second .005 gramme was sufficient. Thus twice the amount of toxine gave rise to a serum 100 times stronger. Such experiments indicate that the process of antitoxine production resembles a process of hypertrophy and bears out the idea that certain cells get into the habit of producing it in greater and greater degree.

(4) *We now come to look at certain difficulties which may arise in a careful consideration of Ehrlich's theory.* The chief may be stated as follows. Suppose an animal is being immunised to a very high degree, for the purpose of obtaining a very strong antitoxic serum such as is used in the therapeutic applications of this substance. Suppose that it has reached a stage when its serum contains an enormous number of

antitoxic units. The process of immunisation is being proceeded with by injecting a fresh dose of toxine. As often occurs in actual practice, this amount of toxine could be neutralised by a very small fraction of the antitoxine already free in the animal's blood. If it be injected hypodermically, then, as it is slowly absorbed into the blood, it must meet an enormous overplus of antitoxine by which it must be neutralised. How then does it ever reach the site where it is to stimulate the receptive cells to fresh activity, in, for example, the case of tetanus, where, according to Ehrlich, the brain seems to be the chief site of fixation? It is true that, in certain animals, a portion of it might be fixed locally at the point of injection and stimulate antitoxine formation there, but to produce the high effects of a long immunisation the toxine would always require to be injected into the same place, and it is found that this is not necessary. But, supposing that it has to reach, say, the brain, in order to effect the purpose of its injection and that it does do so. It is fixed there, but the cells in which it is fixed are constantly bathed with fluids extremely rich in antitoxine. Why then is it not turned out of the cells? That such an eviction ought to take place we must admit if we consider the rationale of the antitoxic treatment of such a disease as diphtheria. We have seen, from the experiments of Dönitz, that if toxine be injected, followed by the injection of antitoxine, the longer the interval between the injection of the two substances, the greater has the amount of antitoxine to be, if death is to be prevented. To save life many thousand times the amount of antitoxine sufficient to neutralise *in vitro* the amount of toxine in the body has to be administered. All this points to the therapeutic action depending on what is called mass action although how the matter can be put into the language of physical chemistry might be difficult to say. At any rate; the therapeutic action of antitoxine seems to depend on the toxine being turned out of its combinations in cells by an overwhelming amount of the anti-body. Now why should the same action not occur during immunisation, in the circumstances we have cited, and, if it did, would not all possibility of fresh antitoxine formation come to an end? We have to face the fact that it does not. There is one possible explanation which is still consonant with Ehrlich's theory. Assuming that, in the case we are supposing, the toxine must reach the brain and be fixed there. Let us consider what would be the effect of the affinity of the brain cells for the toxine being very slightly greater than the affinity of the free antitoxine of the blood for the toxine. The antitoxine in the blood might saturate the toxine which had been

hypodermically injected, and, the combined substance, circulating in the blood, would probably come into contact with brain cells. The more powerful affinities of the latter would break up the weak compound and retain the toxine, which would then work its action within the cells. But how would this affect the therapeutic action of antitoxine in disease? In this case we would have the toxine firmly fixed in the brain cells which are bathed in an enormous quantity of fluid having a less affinity for the toxine. Now, under certain circumstances known to physical chemistry¹, in such a case a small quantity of a compound of antitoxine with toxine might be formed. Thus, carbon monoxide has a greater affinity for haemoglobin than oxygen has; but, if a mixture of carbon monoxide and oxygen, in which the latter is relatively in excess, be brought into contact with CO-haemoglobin then a very appreciable amount of O-haemoglobin is formed. In the case of the diseases in question, it is probable that the detachment of a very minute amount of the poison from the cells in which it is fixed would be sufficient to turn the balance in favour of the sick individual. But it might be said that by the same process toxine might be detached from the brain of the animal undergoing immunisation. Here, however, the amount of toxine fixed usually amounts to many thousand times the M.L.D. for the animal under natural conditions, and the detachment of a small amount of it would not be likely to interfere with the essence of its effect. Thus, though the mechanism of the development of high degrees of immunity associated with strongly antitoxic sera is very complex, an explanation is not impossible.

There is, however, one aspect of the question which is very perplexing and which may be now raised. We must look more closely into what is meant by active and passive immunity. In the early stages of the immunisation of an animal against a toxine, to what does it owe its immunity? The following experiment opens up this question. In the work on immunisation by means of tetanus toxine modified by hydrochloric acid it has been stated (*vide supra*) that, of the serum derived from some of the members of one series of guinea-pigs, it was found that .5 gramme was necessary to neutralise one M.L.D. of ordinary toxine. A careful calculation⁽³⁵⁾ showed that, in the whole of the blood of the body of such a guinea-pig as that from which the serum was obtained, there could not have been more than enough antitoxine to neutralise two M.L.D. Now a number

¹ For a knowledge of the bearings of physical chemistry on this subject I am indebted to the kindness of my friend, Mr D. Nagel, Fellow of Trinity College, Oxford.

of the other animals of this series were tested by the injection of large doses of unaltered toxine to find out what amount of resistance they showed to the latter. In fact immunity was now judged of not by the antitoxic quality of the serum the animals produced but by the actual amount of toxine the latter were capable of resisting. It was found that they could resist the injection of about 110 M.L.D., though some tetanic symptoms appeared. One, however, succumbed to the injection of 122 M.L.D. The resistance may thus be said to have been somewhere just under 110 M.L.D. It is evident that the animals thus tested cannot have owed their power of resistance to the amount of antitoxine present in the fluids of the body. This observation has been confirmed by other similar experiments. The conclusion is that resistance to a toxine is not necessarily co-related to the possession of antitoxic power in the serum. This is borne out by other facts, such as those brought forward by Behring, to the effect that sometimes an animal will show great power of resistance to toxine without having much antitoxine in its serum. In fact the experience of serum institutes seems to be that sometimes animals are met with which, though easily immunised, appear incapable of producing a powerful antitoxine. On Ehrlich's theory the experiments given above might appear to be explained by supposing that, while the cells had developed the capacity of producing side-chains in great numbers, these side-chains were not yet cast off, and therefore were capable of fixing toxine within the cells. But if this is the case what power is preventing the toxophorous groups of the toxine from having a pathogenic influence? Yet in the series of animals referred to above certainly 66 M.L.D., and probably more, could be tolerated without the slightest symptom of tetanus. The same difficulty as to what becomes of the toxophorous groups is suggested if we consider the later stages of immunisation for the obtaining of therapeutic sera as that immunisation is practised. We have seen there are difficulties, not, however, insuperable, in the way of the toxine getting to the sensitive cells, but new difficulties arise if we have fresh unaltered toxine (such as is usually employed) coming into contact with sensitive cells. In order to stimulate the production of fresh side-chains the toxine must rob the cell of the normal function of those already formed. It can only do so by saturating the latter with its haptophorous affinity. If it does so then according to Ehrlich the toxophorous affinity can work its toxic action. Seeing that, in the injections of late immunisation, thousands of M.L.D.'s may be introduced, it is difficult to understand what becomes

of the many toxophorous affinities which must be fixed in the cells, and fixed in the cells in far greater number than when, say, only one M.L.D. is thus fixed in an unprepared animal. The sequence of events in the development of active immunity is thus far from clear. Such considerations as these just advanced suggest the possibility that the process of active immunisation may be different from the process in passive immunity. This idea had been mooted by Behring, who considers that the immunity of the cell is a thing by itself; for it he suggests the name isopathic immunity, while the other he would denominate antitoxine immunity.

There is another set of facts which must be taken into consideration in this connection. Sometimes in the course of an immunisation, when an animal has developed a serum of considerable antitoxic power, on a fresh injection of toxine being practised, acute symptoms of poisoning occur and death may supervene. This is usually referred to as oversensibility. No explanation of this accident has been offered. It would appear as if the immunity of the cell to the toxophorous groups was lost, and that the fixation of these by some such event as we have just spoken of led to the toxic action suddenly becoming effective.

To sum up our conclusions as regards the sufficiency of Ehrlich's theory to account for the development of immunity against the soluble poisons produced by bacteria, we would say that his views as to the chemical antagonism between toxine and antitoxine, as to the constitution of toxines, and as to the methods by which these produce disease effects, have very great support from the facts known. Further, the fixation of toxines in the cells of the body and the genesis of antitoxine from an over-production of some product of cellular activity, are very probable, but the theory does not give a complete account of what takes place in the course of the rise of active immunity. It however accounts completely for the events of passive immunity and for the therapeutic applications of antitoxic sera. It may be here said that what will be the event in a case of disease, such as diphtheria or tetanus, arising under natural conditions will probably entirely depend on the amount of a toxine which becomes absorbed, and this last may depend on the capacities of the body to kill the bacteria producing it, in fact on properties which play a leading part in the resistance of the body to the members of the second group of bacterial maladies. Apart from the therapeutic application of antitoxines it is questionable whether recovery from natural disease depends either on active immunity arising or on the development of antitoxine.

B. THE NATURE OF THE CAPACITY OF KILLING BACTERIA AND ITS
RELATION TO THE DEVELOPMENT OF IMMUNITY.

We now pass to the consideration of immunity from the second group of bacterial diseases,—that in which the actual bodily presence of the bacterial cell is necessary for the production of the characteristic pathogenic effects. It must be remembered that we merely take these as types of the general process of bacterial destruction because in them apparently the direct presence of the bacteria is more responsible for the pathogenic effects than the development of soluble toxines. Immunity occurs against such diseases, and as has been said its establishment appears to involve the killing of the bacterium. In the case of many animals a natural immunity exists against many such bacteria, though it can usually be overcome by increasing the virulence of the bacterium, as by passing the latter through the bodies of a series of animals, etc. Acquired immunity can also be developed, both active, by the injection of non-fatal or modified forms of the organism (and it may be said that a very frequent method of modifying the virulence is to kill the microbe by heat), and passive, by the injection of the serum of an animal actively immunised. The latter method is, however, limited in its applications. There are two chief theories that have been advanced to account for the development of this immunity. One, of which the great originator and upholder is Metchnikoff⁽³⁶⁾, attributes recovery from such diseases to the fact that when the bacteria gain an entrance to the body they attract the phagocytic cells of the latter and are englobed, killed and digested by them. In cases of great susceptibility to such microbic action, either the phagocytes are repelled, or the reaction takes place to an insufficient extent,—some bacteria, not being taken up, multiply and cause the death of the animal. Immunity consists in the conversion of a repellent action of the bacteria on the phagocytes into an attractive one, and the gradual strengthening of the latter so that the phagocytic action is able to meet large degrees of infection without the animal suffering. The development of sera, capable of transferring immunity to other animals, has always been a source of great controversy under such a conception, and has constituted the mainstay of a humoral theory, which had rather a nebulous character until Ehrlich extended the observations already described to antimicrobial immunity also. In looking at the question it will be convenient to take first of all certain facts relating to the sera of immune animals, to give Ehrlich's interpretation of these, and then to consider what

relation the phagocytic theory bears to the production of these sera, and, generally, to immunity from the diseases under consideration.

Pfeiffer's reaction and the results of its study. The starting point for all recent work on this "immunity from infection,"—as the action of bacteria, apart from the action of their poisons, is often called,—was the discovery of what is known as Pfeiffer's ⁽³⁷⁾ reaction. A guinea-pig can be immunised against the vibrio of cholera by the intra-peritoneal injection of a small quantity of a culture of this microbe which has been killed by the vapour of chloroform, followed at intervals of a few days by injections of similar quantities of living cultures. If now some living vibrios be introduced into the peritoneal cavity and small amounts of the peritoneal fluid be withdrawn by means of capillary pipettes every few minutes, it can be found by microscopic observation that almost immediately after injection the naturally highly motile bacteria become motionless, and that, a little later, they lose their characteristic comma shape, swell up into round granules, and finally within twenty minutes break up and disappear. This is Pfeiffer's reaction, and it can also be observed *in vitro*, when the bacteria are mixed with the serum of an animal so immunised. This discovery gave rise to much controversy, and its essential significance in relation to immunity is still somewhat doubtful. Out of the controversies, however, there emerged several facts which have contributed to the progress of knowledge. It had been long known from the work of C. Fraenkel and Sobernheim ⁽³⁸⁾ that the bactericidal action of the serum of a guinea-pig immunised against cholera was destroyed by heating for one hour at 70° C. Pfeiffer noticed that if the heated immune serum along with cholera vibrios were introduced into a guinea-pig's peritoneum the usual reaction took place, and from this he deduced that the immunising material was not altogether destroyed by heat but that in some way it affected the animal's organisation and helped it to dissolve the bacteria. Bordet ⁽³⁹⁾, investigating Pfeiffer's reaction, found that the latter returned if to the heated immune serum a little of the serum of an unimmunised guinea-pig were added (such a serum as the latter, to which it will be necessary to make frequent reference, is usually called "fresh serum").

Analogous investigations regarding haemolytic sera. The real significance of these facts was not appreciated until three years later, when a new line of research was followed by Bordet ⁽⁴⁰⁾ which has been of the greatest service in elucidating the whole subject of

immunity against infection. This consisted in the study of the fact first observed by Belfanti and Carbone that if the blood corpuscles of a rabbit be injected into a horse, after the fashion of an immunisation experiment, the serum of the latter develops poisonous properties towards rabbits, and these properties consist in the fact that on the serum being injected a dissolution of the red blood corpuscles of the rabbit takes place. Further, this phenomenon occurs when the serum is brought into contact with the corpuscles in a test-tube. The method of carrying out the latter experiment is to bleed a rabbit, whip the blood so as to defibrinate it, make up a 5 per cent. solution of the defibrinated blood in .75 per cent. sodium chloride solution, and treat small quantities of this solution (which of course contains the red corpuscles) with the serum. The mixture is allowed to stand a few hours at 37° C. and the occurrence of the haemolysis is indicated by the whole fluid becoming stained by the dissolved-out haemoglobin. Bordet, applying the fact already observed by him regarding Pfeiffer's reaction, found that the haemolytic serum lost its properties by heating for half-an-hour at 55° C., but that, on adding to this heated serum some serum from an unimmunised guinea-pig, the haemolytic action was once more evident, though the fresh serum by itself had no haemolytic properties. On these facts and on others obtained by himself and Morgenroth, Ehrlich⁽⁴¹⁾ based an extension of the antitoxine theory to account for the facts of immunity against infection. According to this view there are two bodies concerned in the process of haemolysis just described. One of these, that which is susceptible to heat being destroyed by half-an-hour's exposure at 55° C., is present in fresh guinea-pig serum. This he calls the "complement." The other is a body which is developed in the guinea-pig serum by the process of immunisation which the animal has undergone and withstands heating for half-an-hour at 75° C. without being entirely destroyed. This he calls the "immune body¹." In the latter there are two haptophorous groups, one of which is satisfied by a receptor in the red blood cell analogous to the receptor which fixes such a body as tetanus toxine in the brain cells of a susceptible animal. The other

¹ Considerable confusion arises through the variety of terms applied to the "complement" and "immune body." Complement is often called by Ehrlich "addiment" and by the French school, constantly, "alexine." By the latter the immune body is called "*la substance sensibilatrice*." All through this paper we have used the terms complement and immune body. As we shall see substances analogous to the latter sometimes occur in ordinary sera. These Ehrlich calls "*Zwischenkörper*," which we have translated "go-betweens."

haptophorous group of the immune body is satisfied by being linked to a corresponding group in the complement. In the latter there is a group analogous to the toxophorous group of the tetanus toxine and this is the active haemolytic agent. The complement can thus only act when, through the intermediary of the immune body, it is anchored to the red blood cell. The experimental evidence on which this theory rests is as follows: If a goat be treated with repeated doses of sheep's blood there develops in its serum the capacity of dissolving sheep's red blood corpuscles (it may be here said that a very great number of similar haemolytic sera can be obtained by treating one species of animal with the blood of another species). Ehrlich took 4 c.c. of 5 per cent. defibrinated sheep's blood in .75 per cent. salt solution, added 1 c.c. of immune goat's serum which had been heated half-an-hour at 55° C. (and which thus contained only immune body), and placed the mixture for 15 minutes at 40° C. The question of where the immune body was he now investigated in the following ingenious way. The mixture was centrifugalised till all the corpuscles were deposited at the bottom of the tube. The supernatant clear fluid was decanted and there was added to it .2 c.c. of ordinary sheep's blood (containing, therefore, susceptible red corpuscles) and .8 c.c. of "fresh" goat's serum (containing, therefore, goat's complement). The mixture was placed at 37° C. for two hours without any trace of haemolysis occurring. Now, if the immune body had been left in the fluid after centrifugalisation, the complement from the fresh goat's serum ought by it to have been linked to the sheep's corpuscles added, and haemolysis of the latter ought to have occurred. The immune body was therefore not here. The sheep's corpuscles of the original mixture were, of course, in the deposit separated by the centrifugalisation. This was now taken, stirred up with 4 c.c. of .75 per cent. salt solution, and there was added .8 c.c. of fresh goat's serum (containing, of course, complement). The mixture was placed at 37° C. for two hours and at the end of this time there was found to have occurred haemolysis of the corpuscles. During the 15 minutes that the original mixture was kept at 40° C., therefore, the immune body in the immune goat's serum had united itself to the sheep's red corpuscles, as was evidenced by the fact that when the latter were exposed to fresh complement haemolysis occurred. As remarked above, complement cannot cause haemolysis by itself. The method of the above experiment is that three factors are necessary to the occurrence of a given haemolysis,—the red blood cells to be acted on, a body resistant to heat occurring in

the serum of the immune animal (immune body), a body susceptible to heat occurring in the serum of an unimmunised animal (complement). When the presence of any one of these substances is suspected, it can be traced by adding the other two and observing whether haemolysis takes place. To proceed,—in the investigation, sheep's blood was next taken, fresh goat's serum was added, the mixture centrifuged, and the fluid on the one hand and the deposit on the other investigated for complement. None was found in the deposit but it was found in the clear fluid, so that no combination had taken place between it and the blood corpuscles. Next it was found that there was a greater affinity between the immune body and the blood corpuscles than there was between it and the complement. The proof was as follows:—It was observed that in a mixture of 5 c.c. of 5 per cent. sheep's blood, 1 to 1·3 c.c. of heated immune goat's serum, and ·5 c.c. of fresh goat's serum, there was just enough of all the constituents to satisfy all the affinities and leave none over. If all these substances were mixed at 0° C., and the mixture centrifuged as before, it was found that the complement present was still free in the supernatant fluid. Therefore the affinity of the immune body for the blood corpuscles was greater than its affinity for the complement. This last experiment also shows that at 0° C. the complement and immune body must have existed free, side by side.

Application of these facts to the explanation of immunity against infection. We must now look at the relation of these facts to a theory of immunity from bacterial infection. First of all here, with regard to the meaning of Pfeiffer's reaction, we have to observe that perhaps its most significant presentation lies in what happens when the heated serum of an immune animal is injected along with cholera vibrios into the peritoneum of an ordinary guinea-pig. If these vibrios were injected alone they would cause the death of the animal, but when they are accompanied by immune serum nothing happens except their own death and solution. Taking this along with the parallel experiment of Bordet conducted *in vitro* we would suspect that, in the intraperitoneal killing of the bacteria that occurs, two substances are at work, one developed in the body of the animal which has been immunised, and the other existing normally in the body of every guinea-pig, and that these by their union effect the death of the organisms. This is in fact Ehrlich's theory and the inference just drawn as to the mechanism of the process is borne out by all his experiments on haemolysis. In the latter, to which so much attention

has been paid because they are more convenient to perform and on the whole are more likely to give accurate results, we have only to read "solution and death of bacteria" for "solution of red blood corpuscles," and the altered conclusions, so far as we know, would be perfectly justified, for all the numerous researches on the subject lead to the opinion that the bodily capacities at work in haemolysis are identical with those concerned in bacteriolysis and bactericidal action. It must, however, be clearly understood that, while the processes are the same, it does not follow that the substances which cause haemolysis are identical with those which give rise to bacteriolysis. This is a point which will be considered later on. With regard to the details of the processes it will be convenient to look at present simply at what Ehrlich would say takes place when blood corpuscles are injected after the manner of an immunisation for the purpose of obtaining a haemolytic serum. The corpuscles first injected are foreign bodies. They have in them affinities which are satisfied by receptors in some cells of the body. The question of the cells in which these receptors occur and of the receptors being set free in the serum will be discussed later. These receptors are of a more complicated character than the receptors of which we have hitherto spoken, for they contain two unsatisfied affinities. One of these can be satisfied by the group in the blood corpuscle. The other is satisfied by a group belonging to a substance which exists in the serum of the animal and which is the complement already referred to. The latter thus becomes fixed to the blood corpuscle, and then through its free affinity,—that which would correspond to the toxophorous group of the diphtheria toxine,—it has a haemolytic action. The side-chains or receptors of the cell which act as a go-between between the corpuscles and the complement have a normal function in the body, and therefore the process of immunisation robs the cell of what it requires for its normal metabolism, just as in the case of the other diseases we have considered; these receptors are replaced by the cell, and by and by, as in the previous case, are over-reproduced beyond the requirements of the body. They are then cast off into the blood stream and form the immune body present in the serum of the immunised animal. If we substitute the word bacterium for blood corpuscle in the above description we have the theory as it applies to bacterial immunity. A question which we shall at present leave over is how the theory applies to the case of natural immunity and to recovery from disease. We may say at once, however, that all are agreed that the two substances named are the essential

factors in the killing of bacteria within the body. The questions at issue are, Are they the only factors? In how far do they exist naturally in the body and where? Where, in any case are they formed? and, Where do the processes of solution take place?

Before we proceed to the consideration of the answers to these questions it is convenient to look at certain facts which have been observed relating to the capacity of the body to affect, and it may be dissolve foreign bodies. Though the fate of red blood cells when introduced into the body of another animal had been long known and in fact had been made one basis for the giving up of the transfusion of actual blood in surgical practice, it was not till after the work of Bordet and Ehrlich that much attention was paid to the occurrence. It was then suggested to various observers to enquire what was the fate of other kinds of cells when these were injected into the bodies of animals other than those from which they were derived. Such researches have been widely carried out under Metchnikoff's instigation. As loss of motility in bacteria, when these were injected into an animal, was a very prominent feature, it was natural to enquire what happens in the case of such motile cells as spermatozoa, and this has been investigated by Landsteiner⁽⁴²⁾, Moxter⁽⁴³⁾, Metchnikoff⁽⁴⁴⁾, and Metchnikoff⁽⁴⁵⁾. The general result may be said to be that if emulsions of the testicle, or if spermatid fluid, be injected into the peritoneal cavity of such an animal as the guinea-pig after the manner of an immunisation, the serum of the animal develops the capacity of immobilising fresh spermatozoa. Metchnikoff has shown that this property is lost if the serum be heated, but such inactivated serum can be reactivated if the serum of a fresh unimmunised guinea-pig be added to it. Generally speaking, however, there is no solution of the actual protoplasm of the spermatozoon. Similar experiments have been conducted with leucocytes by Metchnikoff⁽⁴⁶⁾, Funk⁽⁴⁷⁾, and Besredka⁽⁴⁸⁾. In these, emulsions of spleen, bone-marrow, and mesenteric glands have been injected into guinea-pigs and rabbits and sera have been obtained which have had the power of dissolving white blood corpuscles both *in vivo* and *in vitro*. When injected into the living animal these have the power of giving rise to very pronounced toxic symptoms, due to the great destruction of the cells which takes place, and which, in the case of the peritoneum, can be watched occurring by withdrawing small portions of the exudation by capillary tubes. Such sera are, however, according to Besredka, much more susceptible to heat than the haemolytic sera and entirely lose their toxic actions at 55° C.

According to this observer also, if small non-fatal doses be administered an increase of the number of leucocytes can be observed, and he thinks that a stimulation of the blood-forming mechanism occurs. It is said that a serum produced by the injection of spleen emulsion has a solvent action on the mononucleated and also on the poly-morpho-nucleate leucocytes, while a serum produced by the injection of the bone-marrow has a special action on the latter only. This would be largely explained by the modern view that the marrow is the chief site of the formation of these poly-morpho-nucleate cells; the material used in the immunisation would thus be specially rich in such cells. Von Dungern⁽⁴⁹⁾ has studied the effects of injecting into the peritoneal cavity of the guinea-pig ciliated epithelium derived from the trachea of the ox, and has found that the cells, while being preserved for days in this situation, gradually lose their motility. If now a second injection of the cells from the same source be practised, the latter lose their motility sooner, and this is due to the development of an immobilising serum, which also has a similar action *in vitro* though to a less extent. Again Delezenne⁽⁵⁰⁾ has investigated the effects of injecting into rabbits and dogs emulsions of liver cells and has found that in such animals there develops a serum which possesses a highly toxic action. When it was injected into the animals of the species from which it was derived it produced a condition allied to what occurs in phosphorus poisoning, *i.e.* an acute fatty degeneration of the hepatic cells. Similar poisonous sera have been obtained by the injection of kidney cells and cells from the central nervous system,—the sera in each case acting on the cells of the organs which stimulated their formation. Moxter, working with spermatozoa, and von Dungern in his experiments with tracheal epithelium both noticed the remarkable fact that the anti-sera obtained possessed haemolytic properties.

Such facts, taken along with what we have said regarding haemolysis and bacteriolysis, point towards the conclusion that in the latter phenomenon, with which the subject of immunity has to do, we are dealing with only one example of some great general process which may represent one aspect of normal metabolism. The feature of this process which first arrests attention is the extraordinary complexity of the substances which play a part in it. Evidently the breaking up of foreign cells in the body very usually is dependent on the development of two substances. In one group there is a capacity of resistance to moderate heat, in the other there is not, for at a temperature of 55° C. or thereabout they are destroyed. While these substances have such

features in common, the question arises whether every one is not specific in its action. With regard to the immune bodies all are agreed that this is the case. That which acts on cholera vibrios will not act on typhoid bacilli, and so on. From the standpoint of how these bodies according to Ehrlich's theory originate it must therefore be supposed that, corresponding to each, there was originally a side-chain in a cell capable of being saturated in a particular way and in no other, and from one such group of side-chains each immune body originates. But as we shall see presently the question may be even more complicated, for it is a question whether in many examples of one lysogenic effect several immune bodies may not be concerned. In addition to the complexity of the immune bodies there may also be a complexity of complements, though this is a matter under dispute.

With regard to this last question it may be remarked that while, where an animal is treated with the blood of another, there appears in its serum both immune body and complement adequate to dissolve the blood corpuscles of the species of animal whose blood was used in the immunisation, it does not follow that the immune body developed cannot link on to the susceptible blood cells a complement present in the serum of another species of animal. It is a very common experience when an immune serum has been inactivated by heat that it can be reactivated, not only by the addition of serum from an unimmunised animal of the same species, but by fresh serum derived from another species. This fact as we shall see may have an important bearing on the therapeutic uses of bactericidal sera. There is another point regarding immune sera which may be mentioned, namely, that in them the immune body and the complement are not formed in equivalent proportions. This is shown by the work of von Dungern⁽⁵¹⁾. In rabbits immunised with ox blood a certain quantity of the immune serum dissolved a certain amount of ox blood, but if to this quantity there was added fresh blood from an unimmunised rabbit, *i.e.* which contained only complement, 32 times the original amount of ox blood could be dissolved by the same amount of serum. There was thus present in the immune serum much more immune body than could be utilised by the animal on account of the fact that there was a deficiency of complement. This we shall see is a fact of very great importance. Following up the observation, von Dungern notes that, by taking advantage of it, the amount of complement and of immune body in a serum can be measured. For estimating the amount of complement he takes as a standard the amount of immune serum inactivated by heat which can,

when saturated with complement, dissolve the corpuscles in 8 c.c. of a 5 per cent. solution of ox blood in .8 per cent. sodium chloride solution. Applying this method he states that nearly all rabbits have the same amount of complement, though, as Walker⁽⁵⁶⁾ has pointed out, von Dungern's figures showed that sometimes there might be twice as much as at others. The complement content of immune sera can be obtained by comparing the haemolytic action of the serum in the fresh condition with its action after being heated for 20 minutes at 56° C. Von Dungern states that in rabbits no difference in the amount of complement present in the serum could be detected during the 11 days succeeding the injection of blood. Apparently, however, only the effect of one immunising dose was studied. Thus, though von Dungern's results are not above criticism, there is no doubt that they open up the way to what, as we shall see, is a very important field of research in this subject. Of course for each serum studied in this way an artificial standard of reference would require to be set up.

(To be continued.)

NOTE. The bibliographical references will be given at the conclusion of the second article.

A REVIEW OF CURRENT THEORIES REGARDING IMMUNITY.

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Continued from page 250.

II.

The question of the complexity of immune bodies and of complements can be best illustrated by bringing forward certain experiments of Ehrlich, who has always held to the multiplicity of both substances. These experiments have, like the former, to do with haemolysis.

In immunising two goats with a sheep's blood, Ehrlich found that the serum on being heated for $\frac{3}{4}$ hour at 56° C. lost a capacity which the serum of the normal goat possesses of dissolving rabbit's blood. Only after 3 hours at 56° C., or a $\frac{1}{2}$ hour at 65° C. did the serum lose its effect on sheep's blood. There was thus evidence of a thermolabile and a thermostable complement existing in the same serum. Evidence of multiplicity of immune bodies and complements is also present in the following. Goat's serum dissolves rabbit's or guinea-pig's blood corpuscles. This property is lost by heating to 55° C. but returns on the addition of horse serum, *i.e.* a body exists normally in the serum of the goat which corresponds to an immune body and this finds a sufficient complement in the serum of the horse. It may be said that the horse serum has by itself no haemolytic action on rabbit's blood. Ehrlich proceeds to ask, Are the bodies which dissolve the rabbit's and the guinea-pig's corpuscles the same? First of all are the bodies which correspond to the immune body the same? (When such occur in normal sera Ehrlich calls them "Zwischenkörper," which we shall translate "go-betweens.") Here the amount of inactive goat's serum which by the action of a given amount of horse complement could dissolve a given amount of guinea-pig or rabbit blood corpuscles was determined. The amount of inactive serum was about

the same for the two kinds of blood. The given amount of rabbit's blood was taken, the given amount of goat's serum, inactivated by heat so as to destroy the complement and leave only go-between, was added, and, after standing, was centrifugalised. The clear fluid was now tested for a go-between that would with the aid of horse complement dissolve rabbit's blood. None was present, but on the addition of horse complement to the deposit haemolysis took place. The whole of the go-between between rabbit's blood corpuscles and horse complement was thus attached to the corpuscles. If a second quantity of the rabbit blood was now in the same way treated with inactive goat serum and centrifugalised, and to the clear fluid guinea-pig blood and horse complement was added haemolysis occurred. Haemolysis also occurred when horse complement was added to the deposit. This showed that the rabbit corpuscles, while taking up all the go-between concerned in their own complete solution left something behind which could act as a go-between between guinea-pig corpuscles and horse complement. In other words, in the original amount of inactive goat's serum which was sufficient when supplied with horse complement to dissolve either guinea-pig or rabbit corpuscles there must have been two bodies, one capable of linking the complement on to the one kind of corpuscles, the other capable of linking it to the other corpuscles. There were thus two *Zwischenkörper*. Were there also two complements, one for each go-between? To determine this the amount of normal serum from an unimmunised goat necessary to dissolve the corpuscles in 2 c.c. of a 5 per cent. solution of guinea-pig blood on the one hand and of rabbit blood on the other was found. Such ordinary fresh serum was filtered through a particular kind of filter; it was now noticed that while the amount required to dissolve the guinea-pig blood was the same as before, so far as rabbit blood was concerned it was much weaker. If, however, to this weak filtrate horse complement was added its former power returned. This showed that the reason of the weakening was that some complement had been removed by the filtration and that there was as much go-between as before. In other words, there was evidence that here there were two complements as well as two go-betweens. Ehrlich found the same to be the case with the go-betweens of dog serum and guinea-pig corpuscles. Such serum can be activated, *i.e.* can be supplied with complement either from guinea-pig serum or horse serum.

The relation of these experiments not only demonstrates Ehrlich's ideas as to the complexity of the haemolytic process, but it serves to

accentuate the probability that the relations between the different bodies involved are of a chemical nature. This is not the view taken by Bordet⁽⁵²⁾. According to the latter, while there is no doubt that the immune body is specific for each case of haemolysis, *i.e.* the serum of a goat treated with sheep's corpuscles would not dissolve any corpuscles except those of the sheep, yet there is not evidence of a chemical reaction between the immune body and the appropriate complement. Bordet thinks that the former sensitizes, as it were, the corpuscles and enables a complement to enter into them and cause the actual solution. The main point with this observer is that once a red blood corpuscle is sensitized by an immune body it is liable to a kind of ferment action on the part of different complementary bodies. He brings forward several facts in support of this view. He finds that of a particular haemolytic immune serum from the guinea-pig .4 c.c. could dissolve .5 c.c. of a given solution of rabbit's corpuscles, but if, to such a quantity of the serum, the solution of corpuscles was added gradually (say, .2 c.c. followed in an hour by .1 c.c.), then no solution took place of the blood added after the first fraction. He compares this phenomenon to the taking up of dye by blotting-paper. In a given solution of dye there may be quite enough to dye a large piece of such paper, but if this be torn up and a small piece be placed in the dye, followed at intervals by other small pieces, it will be found that the pieces of paper added last will not be stained so deeply as those placed earlier in the dye. Ehrlich admits the fact regarding the immune serum, though he has in repeating the experiment rather varied it. He determined the exact amount of goat's serum inactivated by heat, which, with the aid of the minimum amount of complement derived from the goat or sheep (both of which complements happened in this instance to be effective), would cause complete solution of 2 c.c. of a 5 per cent. solution of dog's blood corpuscles. To each of a series of tubes, containing the latter amount of dog's blood, there were added different multiples of this simple dissolving dose of inactivated serum, thus $1\frac{1}{4}$, $1\frac{1}{2}$, $1\frac{3}{4}$, 2, $2\frac{1}{2}$ times the amount were added. These stood for 1 hour at room temperature,—no haemolysis occurred for no complement was present, though of course during this time immune body would be taken up by the corpuscles. They were then centrifugalised, and to the clear fluid there was in each case added the same amount of blood and also sufficient fresh complement. It was found that only in the tube, to which 2 c.c. or more of the inactive serum had been added, did haemolysis take place, *i.e.* these were the only tubes in which there was left free

enough or more than enough immune body for complete solution. Therefore no more immune body than was required for the solution of the given amount of corpuscles had been in the first tubes taken up by the latter; in another similar experiment, however, in which another immune serum was used, it was found that the corpuscles had taken up 100 times the amount of immune body required for solution. In other words, in such an experiment as that last described it was not till 100 times the simple dissolving dose was added to the mixture of immune body and blood corpuscles that evidence of any immune body remaining unattached was found in the fluid from which by centrifugalisation the corpuscles had been removed. Ehrlich explains these divergent results by supposing that there are differences in the capacities of different corpuscles to take up immune body. The rationale of these differences he thinks is, that in certain blood corpuscles there may be receptors, *i.e.*, affinities capable of being satisfied by taking up a haemolytic immune body, other than are concerned in the fixation which results in haemolysis. Bordet, from his point of view, has brought forward other experiments on this subject. In the case of the guinea-pig treated with rabbit blood, a serum is obtained which, when inactivated by heat, can be reactivated not only by the fresh serum of the guinea-pig but also by the similar serum of the rabbit itself. Bordet's interpretation of this observation is that the blood corpuscles sensitized by the same immune body are capable of being dissolved by different complements. Ehrlich has repeated the observation and confirmed the facts, but pursuing investigations along the lines of the experiments given above, by which he proved the presence in one immune serum of two immune bodies each of which was capable of saturation by a special complement, he holds that here also there were present in Bordet's serum two immune bodies, and therefore in his opinion he establishes the presumption that here also these were satisfied by two complements, one derivable from the fresh serum of the guinea-pig, the other from the fresh serum of the rabbit.

This brings us to a point of great importance, and one which bids fair to be the cause of considerable discussion, namely, the question of the specificity of immune bodies and of complements. With regard to the former there is, as has already been remarked, very little dispute as to their specificity in relation to any particular haemolytic or bacteriolytic reaction. But in the broad view of the theory of immunity we must clearly bear in mind the limits of this specificity. It must be remembered that the immune body is supposed to be derived from side-chains which

play a part in the normal metabolism of the cell. Therefore we must assume that these side-chains are capable of saturation by other affinities than those possessed by such foreign bodies as bacteria, blood cells, etc. It is quite conceivable that in such normal metabolism these side-chains might be saturated and over-saturated by some material, say, some normal food-material of the cell, and that, through this, there might be cast off into the serum a body which would be identical with the body produced by the saturation of the same side-chains by some foreign body. Such an occurrence would explain the presence of immune bodies in ordinary normal serum, of which we have already seen one example. So far then is the specificity of the immune bodies limited. The main controversy will, however, evidently take place over the specificity of the complements. Is there, as Ehrlich holds, normally existing in the serum of each species of animal a whole series of complements or is there only one? As long ago as 1888 Nuttall, followed in 1892 by Buchner⁽⁵³⁾, had attributed the property, exhibited by many sera, of killing bacteria to the presence of substances which the latter observer called alexines and which Nuttall found were destroyed by heating at 55°C ., and these, if not identical with what are now referred to as complements, belong probably to the same class,—in fact Bordet calls his complementary bodies alexines in recognition of this relation. That one immune serum when inactivated by heating at 55°C . can be activated by different fresh sera is undoubted, but, as we have seen, Ehrlich attributes this to the existence of a series of immune bodies in the same immune serum, each of which acts in conjunction with a corresponding complement. The chief upholder of the non-specificity of complements is as we have seen Bordet⁽⁵⁴⁾, who, while admitting that the complements from different species of animals may exhibit differences, yet apparently thinks that the difference is of a very subtle nature, regarding which our total ignorance of the essential structure of the bodies leaves us entirely in the dark. That they are different he sees he must admit because of this fact;—against these bodies as against toxines there exist anti-bodies which can be produced by injecting the sera containing them into animals. Now suppose we have the case where, as we have shown above is possible, we have an immune body which can be made active against the blood corpuscles which stimulated its formation, by two fresh sera, *i.e.*, by complements *A* and *B* each derived from a different species of animal. Suppose, further, that by the injection of *one* of these sera, say that containing *A*, into an animal, after the manner of an immunisation, we obtain an anti-serum which is capable of neutra-

lising the complement *A* present in the serum injected. In other words, this anti-serum will be able to protect the corpuscles against the serum,—will take away from the serum the property of activating the immune body which is ready to attack these corpuscles. The important fact which indicates that the two complements are not the same is that this anti-serum will be found incapable of preventing the immune body being activated by the other complement *B*. Seeing that Bordet is an opponent of the reaction of immune body, complement, and corpuscle being of a chemical nature and looks on the sensitizing of the corpuscle to the action of the complement by the immune body as being more probably of the nature of the preparation of the corpuscle for a ferment action, it would be quite fair to take as an analogy for his views of the nature of the differences between different complements the differences which exist between the peptic and pancreatic ferments, both of which have a proteolytic action, though each is distinct from the other. While according to Bordet some kind of difference must therefore be admitted to exist between different complements they have this common property, namely, that given a blood corpuscle or a bacterium sensitized by its appropriate immune body a whole series of complements is then capable of entering in and causing solution. Thus, Bordet believes that not only can the blood from two species of animals each sensitized by its appropriate immune body be dissolved by the same complement but that the same complement can also dissolve the sensitized bodies of bacteria. Some of the experiments on which these views are based may be given. A haemolytic serum *A* was obtained by treating a guinea-pig with the blood of the rabbit; another haemolytic serum *B* was obtained by treating a rabbit with the blood of the fowl. In each of two tubes *X* and *Y* the following mixture was made. Of *A* .2 c.c. was taken (this therefore contained immune body capable of dissolving rabbit corpuscles *plus* guinea-pig complement). There was then added 1 c.c. of *B* which had been heated to 56° C. (containing therefore only immune body capable of dissolving fowl corpuscles). To the one such mixture *X* there was added .6 c.c. of defibrinated fowl blood and haemolysis at once took place. This mixture *X* was then allowed to stand some hours. There was now added to both mixtures—*X* and *Y* (to which latter nothing had been previously done)—two drops of rabbit blood. In *Y* the corpuscles of the latter were soon dissolved while in *X* they remained intact. The deduction from this is that the guinea-pig complement present in *X* had been all consumed in dissolving the fowl blood corpuscles. It further shows, according to Bordet,

that the complement present in the mixture was not united to either immune body, but was ready to enter into whichever corpuscle was prepared for it by one or other of the immune bodies. To take another example, which illustrates the taking up of complement by sensitized bacilli. The materials used here were (1) anti-plague serum from the horse heated to 56° C. (therefore containing anti-plague immune body only), (2) serum from ordinary horse similarly heated (containing therefore no active substance), (3) a 24-hour old culture of plague bacilli mixed up in 75 per cent. sodium chloride solution (plague emulsion *infra*), (4) some fresh serum from a guinea-pig (containing therefore guinea-pig complement). Mixtures of these were made as follows:—

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|----|--|
| 1. | ·2 c.c. complement, ·4 c.c. plague emulsion, 1·2 c.c. anti-plague immune body |
| 2. | ·2 c.c. complement, ·4 c.c. plague emulsion, 1·2 c.c. heated horse serum (<i>i.e.</i>
No. 2 <i>supra</i>) |
| 3. | ·2 c.c. complement 1·2 c.c. anti-plague immune body |
| 4. | ·2 c.c. complement 1·2 c.c. heated horse serum (<i>i.e.</i>
No. 2 <i>supra</i>) |
| 5. | ·4 c.c. plague emulsion, 1·2 c.c. anti-plague immune body |
| 6. | ·4 c.c. plague emulsion, 1·2 c.c. heated horse serum (<i>i.e.</i>
No. 2 <i>supra</i>) |

The tubes were allowed to stand for some hours. A guinea-pig had been prepared by the injection of rabbit blood so as to furnish a serum haemolytic to this animal's corpuscles. 2 c.c. of this serum was now taken, heated for half-an-hour at 56° C., so that it now contained only immune body capable of sensitizing rabbit corpuscles, and 20 drops of rabbit blood were added. No haemolysis took place because no complement was present. Of this mixture ·2 c.c. was added to each of the tubes, in fact to each was added a quantity of sensitized rabbit corpuscles. Haemolysis occurred in tubes 2, 3, and 4, but not in the others. Bordet's deductions from this experiment are, (1) that the plague bacillus mixed with the serum of an ordinary horse did not absorb any complement, (2) that the plague bacillus in the presence of the anti-plague immune body of the horse fixed complement of the normal guinea-pig and caused its disappearance from the mixture, (3) that the anti-plague immune body, when plague bacilli were not present, did not unite with the guinea-pig complement. Broadly speaking, no union took place between immune body and complement when these existed apart from sensitive bacilli or blood corpuscles, and further, the same complement was capable of dissolving sensitized

plague bacilli and sensitized rabbit corpuscles. With regard to the proof afforded by Ehrlich as to the multiplicity of complements, based on supposed differences of susceptibility to heat and differences in filtering reactions, Bordet thinks that physical differences may be originated by such processes in different moieties of a substance which is really single. He further submits that different corpuscles present different degrees of sensitiveness to the action of the complement. Gruber⁽⁵⁵⁾, who is an upholder of the non-multiplicity of complements, thinks that Ehrlich's results are to be explained by a failure to take into account different degrees of concentration of complement, and he adduces the fact that while normal ox serum and normal sheep's blood will each dissolve rabbit blood corpuscles, sheep's blood will only do so in very concentrated solution. This criticism has very little point, for in all the researches bearing on the matter the amount of immune body and of complement have been estimated by Ehrlich by the method of von Dungern already alluded to. It may be here noticed that Bordet is of opinion that bacteria when sensitized are more easily affected by a complement than is the case with blood cells. Thus he has found that the cholera vibrio when sensitized by anti-cholera immune body can be dissolved by many different sera. Walker⁽⁵⁶⁾, also, has found that anti-typhoid serum derived from an immune horse can be sensitized by the serum of the rabbit, the ox, and the pig. There is no doubt that the question of whether there exist a large number of complements or whether there is only one such body in each animal species, cannot be at present definitely settled. A settlement can only be looked for from the careful application of the method of von Dungern referred to, and it is fair to say that in the work of Ehrlich and Morgenroth on the subject this has been fully realised.

The therapeutic use of immune sera. There is one point which may here be referred to in concluding this part of the subject, and a point which has an important bearing on immunity so far as the latter is concerned in the process of recovery from disease. It has already been pointed out that, when an animal is being treated for the obtaining of a haemolytic serum, there exists according to von Dungern's observations a much greater proportion of immune body than of complement. Whether during immunisation there is always an absolute increase of complement requires further enquiry. It is, further, a fundamental fact, following from what has been already said, that when a given number of bacteria are to be killed these must be completely sensitized by immune body (if we take Bordet's view),

or completely saturated with immune body (if we take Ehrlich's), in order that they may become amenable to the action of the complement. Further, there must be a sufficiency of complement present to act fully on the bacteria thus saturated with immune body. A deficiency either of immune body or of complement will result in an incomplete reaction and the bacteria will not be thoroughly destroyed. Wassermann⁽⁵⁷⁾, studying the protection which can be afforded to guinea-pigs against typhoid infection by the injection of anti-typhoid serum, found that a case might arise where an animal had been treated with the anti-serum and where after death there might be in its body enough immune body to protect another individual from a fatal dose of the bacilli. The first animal had died because it had not enough complement to utilise the immune body which existed in its blood. Therefore in the use of an immune serum therapeutically, seeing that the latter probably usually contains an excess of immune body, it depends on whether the animal can furnish enough complement additional to that which may exist in the serum to enable it to utilise the immune body injected in the latter. That deficiency of complement is here the danger is further accentuated by the fact that as Walker has pointed out immune serum rapidly loses its complement on being kept. Herein probably lies one explanation of the comparative want of success that has attended the application of anti-sera in the treatment of diseases belonging to the second class which we are now considering. The question of the specificity or non-specificity of complement thus becomes of the highest practical importance. In helping an animal in its struggle against disease it is not only necessary to supply it with immune body in addition to what it may itself be able to manufacture but it is also necessary to see that it is properly supplied with complement. Apparently, the supplying of complement is not such an easy matter as at first sight it seems. It might be thought that all that would be necessary would be to take some of the fresh serum from the same species of animal, which had been used for the immunisation, and inject it along with the immune serum. On this point there seems to be some difficulty. Wassermann states that, if a guinea-pig be immunised against typhoid and the immune serum produced be used for the protection of another guinea-pig against the typhoid bacilli, the serum of a fresh guinea-pig does not supply a sufficient complement. He thinks that in such circumstances the complement either becomes destroyed or is bound in new combinations. He has found that in protecting guinea-pigs against typhoid infection by means of an anti-

serum derived from the dog the best complement is furnished by the serum of the non-immunised horse. Walker has pointed out that some of Wassermann's results depend on a mathematical error, and following this up he has obtained the results regarding the satisfaction of anti-typhoid serum by complements derived from various animals which have been already mentioned. But further investigation is necessary before these results can have a practical application. From what has been said above it will be gathered that Ehrlich does not hold that each immune body has one complement and only one which satisfies it, but he holds that the complements which will satisfy each immune body are probably limited in number, and that the search for such as may be useful in the therapeutic applications of the anti-sera may be attended with considerable difficulty. Even on the supposition that the view which regards the complements as generally interchangeable is correct, the fact that complements derived from different sources may act with different degrees of avidity makes it desirable that the most avid should be used for the purpose under consideration.

There is another matter relating to the therapeutic use of immune sera which is of great importance. It has been shown by Neisser and Wechsberg ⁽¹⁰¹⁾ that under certain circumstances the injection of more than a certain quantity of an immune body derived from another animal may be positively injurious. They have investigated this subject by testing the bactericidal powers *in vitro* of a great variety of sera. In these experiments the amount of complement and the number of bacteria were kept constant while the amount of immune body was varied. It was found that when the latter was present in less than a given amount no bactericidal action was traceable; when it was present in greater amount the bacteria were killed, and again, when there was more than the last amount present, absence of bactericidal action was again noticeable. The explanation which these observers put forward is that if in a bactericidal mixture there be a sufficiency of complement the effect of adding an excess of immune body will depend on whether the effect of the linking of immune body to complement be to diminish or increase the affinity of the immune body for the bacterial cell. If the effect be diminution then the combined immune body and complement will pass the bacteria by, and the latter will therefore remain uninjured. Whether this view be correct or not it is interesting to note that, as has already been shown, Ehrlich found with certain haemolytic sera there was a greater

affinity between the immune body and the blood corpuscle than between the former and the complement. Now in no haemolytic serum has the phenomenon of the injurious effect of excess of immune body been observed. Such facts accentuate not only the difficulty but it may be the danger of the therapeutic use of immune sera in our present incomplete state of knowledge.

To sum up this part of the subject so far as we have gone it is to be observed that the methods by which bacteria are dealt with in the body are similar to those which obtain when many kinds of foreign cells gain an entrance into the latter. The development of artificial immunity against such bacteria depends on the latter being introduced either in a form not strong enough to cause death, or, if virulent, not in sufficient numbers to cause death. In either case, the affected animal probably resists infection because it can develop in its body or already possesses a substance,—immune body,—which attaches itself to the bacterial protoplasm, and in virtue of this attachment permits another body—the complement—which exists normally in the animal's body, to act on the bacteria with a fatal result to the latter. In the case of a further infection with bacteria, such as might occur naturally or as occurs during the process of immunisation, then no illness may result, but a fresh formation of immune body may occur. Whether a fresh formation of complement to any great extent occurs is a question for further investigation, but in an immune serum the complement is always present to a less degree than is the case with the immune body. What the nature of these bodies is is unknown, but the complements are less resistant to heat than the immune bodies. Further, the nature of the reaction which takes place between bacteria, immune bodies, and complement is disputed, and lastly, while the multiplicity of immune bodies is undoubted, it is still an open question whether there are a great number of complements in each animal's body, or whether there is, for each species at least, only one complement which is capable of acting in conjunction with a great variety of immune bodies so as to produce a solvent effect on many different kinds of bacteria.

The sources of the bodies concerned in bactericidal action: the phagocytic theory. It will have been observed that, in what has hitherto been said regarding immunity against bacterial infection, no reference has been made to the source of these bodies, which are found in the serum of immunised animals, and which possess the power of killing and dissolving the offending bacteria, nor has any reference been made to the

site where in the body the death of the latter occurs. It is to be noted that Ehrlich, while holding that the immune bodies are the product of side chains normally present in bodily cells, has never condescended on the cells in which these side chains are situated. Other investigators have put forward theories as to their origin. Chief among these is Metchnikoff, whose phagocytic theory of immunity has been prominently before the world for the past fifteen years. The necessity of co-relating this theory with the facts as to the bacteriolytic properties of sera has now been fully recognised by its founder.

Before taking up this process of co-relation, however, it may be advisable briefly to recapitulate the chief points of the theory in its original form. According to Metchnikoff's view when a bacillus gains an entrance to the body of a susceptible animal, whether it will produce pathogenic effects or not, will depend on whether or not it attracts, does not attract, or repels certain wandering cells present in the body. If such cells come in contact with the bacteria they will englobe, kill and digest the latter, but if this process of phagocytosis does not occur then the bacterium will be free to multiply and will work pathogenic effects. Such a determination of wandering cells towards any foreign material introduced into the body, whatever the nature of such material may be, is of frequent occurrence. The cells chiefly concerned in the process are naturally the wandering cells of the blood, and of these the varieties which have a phagocytic property are the large mononucleated leucocytes and the polymorphonucleate leucocytes, but it is to be borne in mind that these are not the only cells, which, in various parts of the body, are capable of movement and of phagocytosis. In the serous cavities there are many cells, derived in many instances probably from the endothelial lining, which are endowed with these properties, and the same may be true of certain cells, present in connective tissue spaces, which may be derived from the connective tissue corpuscles. In order to clear the ground we may say here that these wandering cells, while the most important, are not the only cells with which Metchnikoff associates a phagocytic property. He recognises a group of what he calls fixed amoeboid cells. Here although the cell is fixed on, say, one side it is free on others and from these can put forth protoplasmic processes and seize on any materials which may be brought into contact with it. Such cells are to be found in the large cells of the splenic pulp and of lymphatic glands, in certain endothelial cells, especially those of small blood-vessels and of serous cavities, in the cells of the neuroglia, and even in certain nerve-cells (in the latter according to Metchnikoff

because of their capacity of taking up leprosy bacilli, which, being non-motile, cannot move into the cells). It is evident, however, that this latter group of phagocytes must play a subordinate part, for such cells can only exercise their functions in this direction when by lymph currents or the blood stream the bacteria are brought in contact with them, or when the latter come into contact with such cells by means of their own movements. It follows from what has been said that the cells taking part in this variety of phagocytosis will depend on the part of the body in which the reaction is taking place.

Of the two groups the free amoeboid cells are thus by far the more important for they can move towards any foreign object. How do they do so? By virtue of this capacity of being attracted or repelled,—the phenomenon of what is known as chemiotaxis. This term was first applied by Pfeffer, and the subject has been studied by many observers, who have dealt with the occurrence as it affects the relations of leucocytes to solid and liquid substances with which they may come in contact. That these cells are attracted by many such bodies there is little room for doubt. The subject has been investigated by Massart and Ch. Bordet⁽⁵⁸⁾ and by Gabritchevsky⁽⁵⁹⁾ by the method of placing in animals capillary tubes filled with the materials and observing whether the cells did or did not wander in. In this way it has been shown that many pathogenic bacteria attract leucocytes under such circumstances. That negative chemiotaxis in the repulsion of bacteria by cells takes place, or at least that leucocytes can exercise a selective effect on bacteria is indicated by the following observations. Disselhorst⁽⁶⁰⁾ studied the effects of applying quinine to the frog's mesentery and found that the leucocytes became round and were rendered immobile and did not migrate as under ordinary circumstances they would have done. On being removed from the vessels it was, however, found that they were not killed but that they now regained their active movements. Jules Bordet⁽⁶¹⁾ stated that in peritoneal infection of guinea-pigs with virulent streptococci no phagocytosis occurred, and the cause of this was not that the leucocytes were paralysed by bacterial action, for if a culture of *Proteus vulgaris* were also injected this bacillus was taken up by the white cells. Zilberberg and Zeliony⁽⁶²⁾ in similar experiments performed with the fowl-cholera organism state that while the phagocytes of an animal do not take up virulent bacteria they still retain their capacity of englobing individuals from non-virulent cultures. There is no doubt that the phenomenon of actual repulsion has been fully substantiated in the case of some of the lower forms of life, especially for some myxomycetes.

With regard to these a further important fact has been established, namely, that an organism which at first shows a negative chemiotaxis, *i.e.*, which is repelled by a substance, may later manifest a positive chemiotaxis, *i.e.*, may be subsequently attracted by the same substance. This possibility of the transformation of the negative into the positive reaction is of great importance in Metchnikoff's theory. In the taking up of foreign substances by phagocytes there has been noticed according to this author a difference in function among the different classes of cells involved.

Roughly speaking the free phagocytes may be divided into two groups, firstly the macrophages which include the large mononucleate leucocytes of the blood and also the large mononucleate cells derived from endothelium, and, in Metchnikoff's opinion, from the large cells lining the sinuses of lymph glands and the sinuses of the spleen. It may be said, however, that Metchnikoff's views on this point are very indefinite, and it is probable that the content of the term phagocyte varies according to the part of the body where the reaction to which it is applied takes place. Differences also may exist between different groups of phagocytes from the point of view of their function. Thus according to Metchnikoff the macrophages are largely concerned in the ingestion of foreign cells such as blood cells when these gain an entrance into the body, while the microphages are chiefly concerned in the ingestion of bacteria. Numerous exceptions to this rule, however, occur and it is by no means certain that the process of phagocytosis can be classified according to such a simple scheme; for instance, as Durham has pointed out and as many other observers have noticed, the microphages may take up foreign bodies and in turn may be taken up by macrophages. The identity of the various cells in the body which are capable of phagocytosis, the processes followed in the course of phagocytosis, and, as we shall see presently, the fate of cells which have exercised a phagocytic action are all subjects which urgently demand further and, above all, unbiassed investigation, for undoubtedly many statements of a too general character have been made regarding them.

According to the phagocytic theory immunity depends essentially on phagocytosis. The recovery of an animal from bacterial infection depends on whether the cells can take up and destroy a sufficient number of the infecting bacteria. Natural immunity depends on the fact that the phagocytic reaction is very pronounced and so effective that an infecting agent is invariably destroyed. The rise of an artificial immunity in an otherwise susceptible animal also depends

on cellular activity. In such an animal under ordinary conditions when infection takes place the phagocytes may either be attracted in insufficient numbers, or be not sufficiently powerful to kill the bacteria, or be unaffected by the presence of the bacteria, or be repelled by the latter. In the process of immunisation the administration of small doses of bacteria or of bacteria in a state of diminished virulence enables the phagocytes to gradually become accustomed to the presence of the latter so that they can ultimately endure and dispose of what under ordinary conditions would have constituted a fatal dose for the animal to which they belong. In the case where ordinarily the phagocytes are repelled, first of all the negative chemiotaxis becomes converted into a positive chemiotaxis and then the same accustoming of the attracted cells takes place.

The adaptation of the phagocytic theory to Ehrlich's observations. If we analyse the process of phagocytosis we see that it divides itself into two parts,—firstly, the attraction of cells by bacteria, secondly, the killing and digesting of the latter by the attracted cells. On the former of these phenomena Ehrlich's work throws little or no light, but Metchnikoff adapts the results of investigations on immune sera to explain what occurs in the latter. According to his new view the immune body and complement are substances produced in the protoplasm of the phagocytes, and it is by means of them that bacteria are killed and digested after being englobed. These substances therefore normally occur in certain cells and may play a part in the digestive activity of these cells. In the phagocytosis which occurs in natural immunity Metchnikoff holds that they never leave the cells and can thus only come into action when the bacteria are taken up by the cells, but in the process of artificial immunisation there is evidence of the immune body escaping into the plasma. There is, however, according to his view no evidence of complement becoming free in the body, and therefore so far as the immune animal is concerned its escape from the bacteria which are injected in the immunisation process depends on these bacteria being taken up by phagocytes and there meeting the complement which is necessary for their destruction. The fact that in immune sera both bodies are found is to be explained by the latter escaping through the breaking up of dying phagocytes or by the phagocytes giving up substances in the process of dying. The resistance of the artificially immune animal to large doses of the infecting agent, like the resistance of the naturally immune animal to ordinary infection, depends on the bacteria being taken up by cells. It is only in passive immunity that

materials present in the serum of immune animals in consequence of the death and disintegration of phagocytes are utilised for an extra-cellular destruction of bacteria in the bodies of the animals to which such sera may be transferred. In immune animals whether the immunity be artificial or natural there is no such extra-cellular destruction of bacteria.

We must now proceed to discuss the evidence which has been brought forward in support of this hypothesis. First of all here we must say that a purely humoral view of the process of immunity is to be put aside as untenable. Such a view would, it may be supposed, rest on the idea that the presence of bacteria in the fluids of the body could cause such chemical changes in these fluids as would make them antagonistic to the life of bacteria. But all changes in the bodily fluids that we know of are traceable to cellular activity, and no evidence can be brought forward that antibacterial action is any exception to the rule. The only modification of such a theory would be that which attributes antibacterial action to substances formed by the bacteria themselves, but at present there is no evidence of the existence of such bodies. Practically then the question which has to be decided is whether bacteria when they gain an entrance into the body are destroyed only by being actually taken up by cells or whether cells can respond to the stimulus of the presence of bacteria to shed forth substances which can kill and digest these bacteria in the fluids of the body. The idea of the possibility of such substances being the active materials in immunity against infection has long been entertained, having been in the first instance suggested by the discovery of the part played by antitoxines in immunity against intoxication by bacterial products.

Bactericidal properties in normal sera. In speaking of these we must strictly differentiate between the bodies of this kind which have been found to exist in the sera of non-immunised animals and those which as we have seen have been found in the sera of immune animals, and we shall first of all treat of the former. Now in 1888 Nuttall⁽⁶³⁾ had pointed out that the sera of normal animals possessed bactericidal properties which were destroyed when the serum was heated at 55° C. Such properties have been found to be possessed by a great number of sera, and by many it was thought that in naturally immune animals the immunity was due to the existence of these properties. Those who adopted such a view overlooked the fact that Nuttall had pointed out that the serum of the rabbit was capable of killing *in vitro* the

B. anthracis, though the animal is susceptible to anthrax, and it was not long before it was recognized that the mere possession of bactericidal serum in an animal was not invariably co-related with the existence of natural immunity. About 1892 Buchner, to whom the above discoveries are usually erroneously attributed (for he only confirmed Nuttall's results), gave to the bactericidal substances in sera the name of alexines. An enormous amount of work has been done with regard to the bactericidal properties of sera into which it is unnecessary from the present point of view to enter. Its result generally speaking may be said to indicate that, as in fact Nuttall pointed out in his original paper, very great differences exist in the action of a given bactericidal serum upon different bacteria. Thus in man Wright⁽⁶⁴⁾, who has made many valuable contributions to this subject and who has designed a method by which bactericidal power can be quantitatively measured, has shown that the serum while bactericidal towards the cholera vibrio and the typhoid bacillus has little action on the pyogenic cocci or on the plague bacillus. Further it may be said that neither is natural immunity necessarily associated with bactericidal power in the serum, nor is the absence of such immunity associated necessarily with the absence of such bactericidal action. It is difficult to explain such facts, but there is one line of investigation which has still to be followed and which may throw some light on the real nature of these natural bactericidal powers. It has still to be shown that the bactericidal action thus naturally present is of the same nature as that which is present in the sera of immune animals. If it is, then probably it might be found that two substances are involved just as is the case with immune sera, and some light might be thrown on this very difficult question of the relation of bactericidal power to natural immunity. That such a line of enquiry might be profitably followed is indicated by what has resulted from the pursuing of enquiries relating to analogous haemolytic actions. Thus Ehrlich and Morgenroth (*loc. cit.*) have shown that ordinary goat's serum will haemolyse the blood corpuscles of the rabbit, and as in the case of an ordinary immune serum this power is lost by heating the serum for half-an-hour at 55° C. Now it is found that in many horses the serum which has in itself no haemolytic action on the corpuscles in question, will, if added to the inactivated goat's serum, cause the latter to regain its haemolytic action. There is thus reason for believing that in the serum of the ordinary goat there are two bodies capable when acting together of dissolving the corpuscles mentioned. One of these—that

which is resistant to heat—corresponds to the immune body found in the serum of an animal which has been subjected to treatment with blood corpuscles,—the other which corresponds to the complement of such an immune serum. In the serum of the horse, on the other hand, there exists, as far as rabbit's corpuscles are concerned, only a complementary body, which, however, when the normal complement is removed by heat from the goat's serum can supply its place. It is quite possible that similar facts may hold with regard to bactericidal action, though the search for them would be of a laborious description. That it is possible that in the bactericidal action of normal sera the same factors are at work as in immune sera is further indicated by certain results of Neisser and Wechsberg (*loc. cit.*). These observers found that just as the bactericidal effect of an immune serum was inimically affected by an excess of immune body, so when a serum naturally possessed bactericidal properties and when at the same time it was capable of supplying complement to an immune body the addition of an excess of the last robbed the serum of its natural bactericidal action. Thus normal guinea-pig serum is bactericidal towards typhoid bacilli. It also has the capacity of replacing the complement which is present in the serum of a dog and which when the latter animal is immunised against typhoid renders the immune body developed active. But if to a bactericidal mixture of guinea-pig complement and dog immune body an excess of dog immune body be added then the bactericidal action is no longer manifest.

In connection with the bactericidal action of normal sera and the relation of the fact to immunity some observations have not long ago been made which are of great importance. Is it certain that the properties of the shed blood of an animal are the same as those of the circulating fluid? This question has been attacked by Gengou⁽⁶⁵⁾ who received the blood of normal (*i.e.*, non-immunised) animals into paraffined tubes. In these no coagulation or only the least degree of coagulation took place, and therefore the fluid which was separated by the centrifugalising process to which Gengou next submitted these tubes was almost unaltered *liquor sanguinis*. Other moieties of the blood were, at the same time, received into ordinary tubes in which coagulation took place as usual and therefore the properties of the ordinary serum could be compared with those of the blood plasma. He found in the bloods he examined (rabbit, dog, rat), that while in many cases the serum showed very definite bactericidal action, the plasma showed sometimes very little, sometimes none

at all. This must be looked on as constituting the most important contribution to recent enquiries as to the nature of bactericidal action. The method must be further applied before the full significance of the results already obtained can be realised and before it can be seen if any light is thrown on the nature of natural immunity. That this is necessary may be judged of by the fact that in Gengou's experiments with the serum and plasma of the dog no attempt was made to investigate the action of these on the *Bacillus anthracis*, to which this animal shows considerable natural immunity, whereas this organism was used with fluids derived from the susceptible rabbit. If the result of an extension of the method be to substantiate the view that under ordinary circumstances no bactericidal power is possessed by the plasma of animals it must be admitted that strong support will be given to the idea that the actual taking up of bacteria by cells is necessary to immunity. On this point, however, judgment must meantime be suspended, for another series of experiments must now be referred to which seem to point to the extra-cellular presence in the *liquor sanguinis* of an ordinary animal of a substance corresponding to a complement and which may play a part in the struggle of such an animal against bacteria to which it is susceptible.

Can complement and immune body occur free in the liquor sanguinis?

It has already been pointed out that when an immune serum is injected into an animal after the manner of an immunisation there are produced bodies which are capable of neutralising its action. Immune serum contains both immune body and complement so that an anti-serum to such might contain an anti-immune body and also an anti-complement. It will be remembered that an immune body inactivated by heat can be reactivated by normal serum from a non-immunised animal. Now if the latter, which contains only complement, be introduced into the body of an animal a serum containing only anti-complement will be obtained. Wassermann⁽⁶⁶⁾ obtained such an anti-complement by injecting the serum of an ordinary guinea-pig into a rabbit. Now the guinea-pig is naturally susceptible to virulent races of typhoid bacilli, and it can be protected against the pathogenic action of these if along with the bacteria some serum from a previously immunised animal be injected. Such an immune serum may or may not contain much complement, for as we have seen an immune serum rapidly loses its complement. If there be any present, its action will be reinforced, and if there be none its place must, if recovery is to take place, be supplied by complement which ordinarily exists somewhere

in the guinea-pig's body. Now if to a dose of bacilli sufficient to cause death there be added enough immune serum to enable the animal under ordinary circumstances to resist such dose, and there be also added sufficient of the anti-complement described above to neutralise the complement which would ordinarily act through the immune body present, then as the complement is neutralised by the anti-complement and as therefore the immune body is in such a mixture of no use, the animal ought to die, and this Wassermann found to be the case. He therefore deduces that complement is not confined within cells but naturally exists free in the guinea-pig's blood. Besredka⁽⁶⁷⁾ criticises these results and holds that what the anti-complement actually does is to paralyse the functions of the phagocytes. This view is based on a comparison hour by hour of what takes place in the peritoneal cavity of an animal injected as above and that of one which received bacilli plus immune serum alone. Unfortunately apparently no comparison was made of the condition in the former with the condition in a case where the bacilli were alone administered and gave rise to the ordinary fatal illness. It might thus have been observed if there was any less evidence of negative chemotaxis in the last case. Without this the experiments lose much of their significance.

We have already said that Metchnikoff admits that in the struggle between an immunised animal and infecting bacteria two substances play a part—the immune body and complement of authors who have investigated the properties of immune sera,—but he holds that while in the living animal the former can escape from the cells where it is formed and be free in the bodily fluids, the complement, on the other hand, always is confined within cells. He apparently is further of opinion that both bodies are formed in the same class of cells, and under ordinary circumstances bacteria are destroyed only when they are taken up by these cells. Thus in a case where a particular species of bacterium is taken up by the microphage cells these would elaborate both the immune body and complement necessary for the manifestation of bactericidal action. The evidence alleged in favour of this view rests very much on a discussion of the phenomena of Pfeiffer's reaction to which attention has already in part been given. In this reaction, as we have seen, when immune serum heated so as to destroy the complement is injected into the peritoneal cavity along with active cholera vibrios the latter are killed and dissolved in consequence of the immune body being activated by complement derived from the plasma of the infected animal. How, if Metchnikoff's view is correct, does this complement

come to be free? This leads us to consider his view of the incidents of a peritoneal infection. When any foreign material,—bacteria, bouillon, etc.—is injected into the peritoneum there is stated to occur within a few minutes of the introduction an apparent almost complete disappearance of the cells,—phagocytes, wandering cells generally,—from the cavity. This Metchnikoff called the stage of leucopenia, but now rather prefers the term phagolysis, and he attributes the scarcity of cells to the fact that they are broken up from being injured by the operation of the injection. This breaking up of the phagocytes, in the case when immune body and cholera bacilli are injected, liberates the complement or “cytase” as he calls it, which acting through the immune body causes the destruction of the bacteria. Considerable attention and great controversy has arisen regarding the precise march of events in the very commonly practised peritoneal infection, and various interpretations have been put on the phase of phagolysis. The general sequence of events is that when bacteria are injected into an animal of moderate susceptibility towards them they very soon begin to be taken up by cells, but within a short time the apparent disappearance of cells from the serous cavity begins. This stage is succeeded by one in which a great influx of fresh cells occurs, which are at first of the order of macrophages, but later microphages predominate. If the reaction is successful free bacteria gradually disappear and the hyperleucocytosis subsides. Durham⁽⁶⁸⁾ holds that the phagolysis is almost entirely accounted for by the fact that within a few minutes of injection there occurs a gathering of the leucocytes into balls which adhere to the peritoneum, especially to the great omentum. Here it may be said that, especially by French investigators (cf. Roger⁽⁶⁹⁾), a much more active function is assigned to this part of the peritoneum than it is usually credited with in this country. It is looked on as a great opened-out gland (*un ganglion lymphatique étalé*) whose cells have proliferative and protective powers, and whose removal renders an animal more susceptible to bacterial infection than usual. This, however, by the way. To resume, Durham denies that there is any evidence of much, if there is of any, disintegration of cells during this period. Pierallini⁽⁷⁰⁾ finds evidence of the shedding of materials by the leucocytes during leucopenia in that, by Weigert's stain for fibrin, strands of this material can be seen on the omentum removed at this stage, but apparently he has not made control preparations of the normal omentum, from which it could be judged if this fibrin formation did not take place during the time occupied in putting up the specimen. He shows, however, that the

mere clumping of the leucocytes has not robbed them of life, for though if removed from the omentum they appear immobile, in the course of a few hours they regain this power and also can be proved capable of phagocytosis. It is evident that the actual disintegration of white cells is not absolutely necessary to the appearance in the blood of substances derived from their protoplasm. Pfeiffer⁽⁷¹⁾ brings forward as evidence against the setting free of material by phagolysis a fact observed by him, namely, that in an exudation very rich in leucocytes his phenomenon takes place rather more slowly than in one poor in these cells. Bordet⁽⁷²⁾ on the contrary states that Pfeiffer's reaction does not take place when the vibrios are introduced into parts of the body of an immune animal where phagolysis cannot take place on account of the poorness of cells. Such parts are the anterior chamber of the eye, the subcutaneous tissues, and the fluids of passive oedema. He says that in the latter the fluid can be made active if along with the bacteria there is introduced some serum from a fresh unimmunised animal. This last experiment is what leads Metchnikoff to admit that in the immune animal immune body can exist free in the plasma. Pfeiffer states that the reaction does occur in the subcutaneous tissue, though more slowly than in the peritoneum. Metchnikoff retorts that probably in Pfeiffer's experiments there was a little subcutaneous bleeding, and that in the process of clotting destruction of some leucocytes would occur and thus complement be liberated. A further proof brought forward by Metchnikoff in this connection is that when the stage of leucopenia is suppressed then Pfeiffer's phenomenon does not occur or only does so to a slight degree. If some bouillon be introduced into the peritoneal cavity of an animal the stages above described take place, but if on the following day the operation is repeated then the stage of leucopenia is suppressed, and if at this time bouillon containing the cholera vibrio and immune serum be injected, then Pfeiffer's phenomenon does not take place, *i.e.*, there is no extra-cellular destruction of the bacteria, such destruction now taking place entirely within the cells. All these considerations lead Metchnikoff to the conclusion that under ordinary circumstances whether or not the bacteria are combined with the immune body within or without the cells it is only within the cells that the final step—the action of the complement—necessary to the bacteriolysis can take place. It must be remembered that the phenomena occurring in a peritoneal infection are as yet by no means clear, for so much depends on the technique practised. This is especially the case when the question of the diminu-

tion or increase in the number of free cells is concerned, for there is no means of estimating the total number of cells present, nor of measuring variations in the amount of fluid exudation. Great caution is therefore called for in forming any opinion from such data as are available. It is evident that the general question of the free existence of immune bodies and of complement demands that further enquiries be made along the lines of the experiments of Gengou on the one hand and of Wassermann on the other.

The sites of formation of complement and immune body. It is evident that in this connection it is important to enquire if any other light can be thrown on the sites of formation of the immune body and complement. The earlier work on the bactericidal actions of normal sera had indicated that the leucocytes were the cells responsible for the formation of the bodies concerned in this process. Thus Denys and Havet⁽⁷³⁾ found that while the whole blood of the dog manifested considerable bactericidal power toward the *B. anthracis* the serum of the same animal had very little action; further, that when from the blood the white cells were removed by filtration through filter-paper the action also disappeared. As showing, however, what care is necessary in making generalisations on the subject, it may be remarked that in testing the blood of man by the same method with the *B. coli* it was found that very little difference existed between the serum and the whole blood. Havet⁽⁷⁴⁾ found that, if *Staphylococcus pyogenes aureus* was injected intravenously in dogs, within a few minutes there was a great disappearance of leucocytes from the blood, and along with this there was a diminution of bactericidal power in the shed blood. After a few hours there was a great increase of these cells above the normal, and there was a corresponding increase of the bactericidal action. In connection with the subsequent discoveries of immune body and complement, some facts observed by Denys and Leclef⁽⁷⁵⁾ are of interest. They immunised rabbits against *Streptococcus pyogenes* and compared the properties of the blood with those of the blood of unimmunised rabbits. They found that the serum of fresh rabbits exercised no bactericidal action on the bacterium in question. The serum of immune animals showed a degree of potency, but not very much. The leucocytes of fresh rabbits mixed with the serum of fresh rabbits had a very feeble action and died before they naturally would have done if no bacteria had been present. The leucocytes of fresh rabbits transported to the serum of immune rabbits destroyed the bacteria and showed a normal degree of vitality. The leucocytes of the immune rabbit when

added either to the serum of immune rabbits or to the serum of fresh rabbits behaved exactly like the leucocytes of fresh rabbits. These results pointed to the fact that for a maximum bactericidal effect two factors were necessary, one present in the immune serum, and the other existing in the leucocytes either of the immune animal or of an ordinary animal, and they indicate that in some way the leucocytes are concerned in the formation of the active bactericidal bodies. Deutsch⁽⁷⁶⁾ states that the anti-body of typhoid serum is specially developed in immune animals in the bone marrow, and that there is little evidence of its presence in peritoneal exudations or in the great omentum. His method consisted in comparing the effects of emulsions of given weights of different organs. As to what weight is to be attached to results thus obtained constitutes a difficult question. In the cases of these bactericidal and protective bodies just cited no experiments have been performed which indicate the source or sources of the two necessary constituents of the reaction. Bulloch⁽⁷⁷⁾ has brought forward more precise observations to show that the development of a haemolytic complement is in the rabbit to a certain extent associated with a rise in the number of certain polynuclear leucocytes in the blood, while on the other hand a development of immune body is associated with the activity of the mononucleate corpuscles. My friend Dr Walker informs me that in the case of anti-bacterial sera he has found that in the first few hours after the removal of blood from the body there is a gradual rise in the amount of complement—this it is certain must come from the white cells present. As has been remarked, the view of Metchnikoff is that both the immune body and complement are formed within the same cells, but that the former is not so intimately bound to (*lié*) the cells as the latter. This would not accord with the results of Bulloch, and the question evidently demands further enquiry. Tarassévitch⁽⁷⁸⁾ has found that emulsions of the organs of the body associated with the formation of the large mononucleate leucocytes (which constitute such a large proportion of the total number of macrophages),—such organs, namely, as the great omentum, the lymphatic glands, the spleen, have a marked haemolytic action on the red blood corpuscles of birds, and that this property is destroyed by heating to 55° C., while it is greatly augmented by the addition of corresponding immune bodies. From a few experiments done it was further observed that the emulsions named had no bacteriolytic effect. On the other hand exudates containing many polymorphs (*i.e.*, microphages) manifested no such haemolytic action even

when abundant immune body was furnished. From this the author deduces that the complement (macrocytase, as he and Metchnikoff call it) for the solution of corpuscles is produced by the macrocytes, and is different from the complement manufactured by the microcytes which is probably concerned in the bacteriolytic manifestations and which is called microcytase.

To speak generally from the rather fragmentary state of our present knowledge, the complement and probably also immune body are produced by the white blood corpuscles, but when we think of the very varied cells which may take on a phagocytic action, we must be careful not to exclude the possibility that they are not the only cells of the body capable of manufacturing these substances. We must also bear in mind that the reaction of the body against infection may not be confined merely to cells which act as actual phagocytes. There are many cells of which the eosinophile leucocytes may be taken as the type which enter into the struggle against an invading agent, and which may discharge from their protoplasm substances having a bactericidal or bacteriolytic function.

The relation of agglutination to immunity. It was long after the promulgation of the phagocytic theory that the very great complexity of the questions involved was realized, and it must now be recognized that very many different processes are involved in the reaction of the body against infection. A very good example of this is found in the process of agglutination of the invading bacteria which is often observed, and to which great attention has been paid from its diagnostic importance in such a disease as typhoid fever. If in a droplet of the serum of a typhoid patient diluted with bouillon there be mixed some typhoid bacilli, these rapidly lose their mobility and gathering together in large clumps gradually also lose their characteristic shape. This clumping occurs under similar circumstances with many other bacilli, and an identical condition often occurs when red blood corpuscles are placed in an immune serum capable of haemolysing them. Further, the reaction is generally speaking specific for a given species of bacterium in relation to a particular serum produced by immunisation with that species, and a certain degree of specificity also exists among races of a particular species. Thus a serum produced by one race of typhoid bacillus will clump that race better than other races (Walker⁽⁷⁹⁾). Sometimes, however, agglutinating properties are possessed by normal sera. The real significance of the occurrence and its relation to immunity are

still obscure, but it is known not to be an essential factor in the action of an immune serum. Thus Fraenkel and Otto⁽⁸⁰⁾ fed young dogs on typhoid bacilli and found their serum was agglutinative, but not bacteriolytic. Pfeiffer and Kolle⁽⁸¹⁾ produced an immune serum which was bacteriolytic but not agglutinative, and Widal and Nobe-court⁽⁸²⁾ often obtained a similar result with mice immunised against typhoid by injecting urine containing the bacilli. Facts of a similar kind have been observed with sera derived from unimmunized animals. Thus the rat, which is susceptible to anthrax, has a serum bactericidal but not agglutinative towards anthrax bacilli; the insusceptible dog has a serum agglutinative but not bactericidal. Further, Trumpp⁽⁸³⁾ showed that while the bactericidal properties of a serum were lost by heating at 55° C. the agglutinative action still remained. It has been observed that several chemical substances such as saffranine if added to ordinary non-agglutinating serum will confer on it agglutinating properties towards the typhoid bacillus. From the fact that the fluids in which anthrax bacilli have been grown have an agglutinating action on fresh cultures the theory has been advanced by Malvoz⁽⁸⁴⁾ that agglutinating substances (often referred to as agglutinins) are really formed by bacteria themselves. Conforming to such a view is the observation of Macrae⁽⁸⁵⁾ that if a collodion capsule containing a fluid culture of typhoid bacilli be placed in a guinea-pig's peritoneal cavity the serum of the animal acquires agglutinative action. Such a phenomenon and also the appearance of agglutination in an immune serum would on this view be explained by the diffusion out of the bacilli of the agglutinating substance. Two other theories presuppose the existence of a special substance in an agglutinating serum. One is that of Gruber, to the effect that the membrane of the bacterium is rendered viscous and this favours adhesion together in masses. This view is also in effect that of Nicolle⁽⁸⁶⁾, who, however, leans to agglutination being the result of two substances, one present in the serum, the other in the bacterium. He finds evidence of the latter having dissolved out in old cultures of—among other organisms—*B. coli*, and observed the remarkable fact that when such a culture was filtered through porcelain and the appropriate serum added to the filtrate an agglutinating process occurred. This agglutinable material in such a filtrate further could be entangled by typhoid bacilli, or even by talc powder, and on addition of the serum to such mixtures agglutination occurred. On the other hand is the view of Bordet⁽⁸⁷⁾, which is that "serum in acting on microbes changes the relations of molecular attraction be-

tween the bacteria and the surrounding liquid." This observer rejects Nicolle's theory on the ground of the following experiment. Cholera vibrios were clumped by the immune serum and the vibrios separated from the supernatant liquid by centrifugalisation and after washing divided into two parts. One of these was treated with .7% sodium chloride solution and the other with distilled water and the deposit shaken. In the former fluid the clumps were re-formed, in the latter the vibrios remained separate. Bordet considers that agglutination may be of the same nature as coagulation chiefly because some haemolytic sera which agglutinate red blood corpuscles also cause a precipitation of the serum to which the corpuscles belong. It must be pointed out however that the haemolytic sera in question were produced by the injection of defibrinated blood, *i.e.*, of corpuscles *plus* serum, and it is now known as will be noticed later that by itself the serum of one animal if injected into another species of animal after the manner of an immunisation will cause that animal's serum to assume the property of precipitating the serum of the first animal. This fact has later been recognized by Nolf⁽⁸⁸⁾. There is no doubt however that there are many facts relating to coagulation, and even to the formation of flocculent precipitates in the chemical reactions of simple inorganic bodies, which must be explained on the lines of changes in molecular attraction such as Bordet lays down with regard to agglutination. On the whole the evidence is rather in favour of some such view as that of Nicolle that for the occurrence of agglutination two substances are necessary, one in the bacterium and one in the immune serum. For one thing, from Nicolle's results, the agglutinable substance of the bacterial cell is apparently more resistant to heat than agglutinins are, and also it is soluble in alcohol, which the latter probably are not. The views of Bordet and Nicolle are not absolutely irreconcilable; the former deals with a process, the latter with the substances concerned in the process. It may here be said that Ehrlich apparently holds agglutination to be due to the formation of special substances in an immune serum, analogous but distinct from those concerned in bactericidal action. This latter fact is the important one in connection with the subject.

The possible part played by ferments in bactericidal action. The question of agglutination has been entered into at some length in order to indicate how complex the problem of immunity is. It illustrates further the possibility of there being many different factors at work in the relation of a phagocyte to the bacteria which it englobes. Metchnikoff seems to attribute both the killing of the bacteria and their

solution to the presence in the phagocyte of a ferment—the cytase—which roughly speaking corresponds to the complement of other authors. If the conception of fermentation has any definite meaning, the term is applicable to the process in which a body possesses the power of originating changes in other bodies while remaining itself unchanged. Of the existence of such bodies there is evidence in the fact that the action of given amounts, of known ferments is indefinite so long as the products of the fermentation are removed. For this idea of ferment action there is often too great a tendency to substitute a proof resting on another attribute of known ferments, namely, susceptibility to moderate degrees of temperature. Very many ferments such as those concerned in peptic and pancreatic digestion lose their fermentative properties at a temperature of 55°C ., but this is just about the point when changes begin to take place in albuminous molecules generally under the influence of heat. And very profound changes may be originated in such molecules by exposure to this temperature. Thus Ramsden⁽⁸⁹⁾ has shown that if egg-albumin be kept long enough at 55°C . almost the whole of it undergoes coagulation. This criterion cannot thus be applied too rigorously to the substances concerned in immunity, though most of them would fulfil it. If we seek to enquire whether the fundamental quality of ferments is recognisable in the case of the bodies under consideration we find the evidence is scanty, if not entirely non-existent. In the case of a toxine such as that of tetanus, as we have seen, all evidence of the presence of the poisonous substance being present during the duration of the disease is absent. In the guinea-pig the poison is anchored in the sensitive part of the body. Again, the definite relations which exist between a definite amount of immune body acting along with a definite amount of complement to produce a certain definite effect, coupled with the facts discovered by Ehrlich of definite linkings taking place in the interaction, is rather against the idea of a body being concerned which originates change without itself being changed. It is further, however, an assumption on the part of Metchnikoff to suppose that the bactericidal and digestive properties of phagocytes are necessarily due to the same substances. That ferments can be formed by leucocytes there is little doubt. Delezenne is stated by Metchnikoff⁽⁹⁰⁾ to have shown that a ferment called enterokynase (which is said to materially assist the pancreatic ferment in digesting proteid) is the product of the lymphoid tissue of the intestine, and the work of Hedin, who has extracted proteolytic ferments from the spleen—the great normal site of leucocyte

destruction—confirms this view. But a ferment action on dead proteid is entirely different from such an action on living protoplasm. The typhoid bacillus will live and multiply in a solution of pancreatic ferment which will digest fibrin. It is thus quite possible that by virtue of one set of powers a phagocyte may kill a bacterium, by virtue of another set of powers it may digest it, and the latter process may be the same as ordinary proteolysis as it occurs in connection with the intestinal glands of an animal.

Fischer⁽⁹¹⁾ has attempted to explain the phenomena of bacteriolysis from a physical standpoint by supposing them sufficiently accounted for by processes of dialysis. Such a view however meets a difficulty in the specificity of immune sera. Further, Bordet⁽⁹²⁾ has brought forward an experiment which seems to indicate that in the case at any rate of blood corpuscles undergoing haemolysis a change occurs which makes the corpuscular protoplasm no longer capable of dialysis. If red blood corpuscles be treated with distilled water the process of "laking" takes place by which they swell up enormously and lose to the surrounding fluid a very considerable part of their haemoglobin. If however some common salt be added, the corpuscles regain to a certain extent their form, and a certain amount of the haemoglobin is re-entangled in the stroma. In the case of red blood corpuscles haemolysed by an immune serum the appearances in the first instance closely resemble those of the corpuscles of laked blood, but the addition of salt has no effect in restoring the structure of the corpuscles. "It appears," to quote Bordet's words, "as if the alexine of the active serum has destroyed, has digested 'a something' in the corpuscle which controls the operations of plasmolysis,—the phenomena of osmosis." In this connection it may be observed that if haemolysis by toxic sera and bacteriolysis are precisely parallel processes it is fair to ask what in the solution of red blood cells corresponds to the killing stage in the case of bacteria. Our general conclusions here must only be that it is advisable not to take refuge behind such an indefinite term as fermentation when in reality nothing is known of the essential nature of the processes concerned.

The nature of chemiotaxis. What we have said hitherto regarding the phagocytic theory concerns only the process by which the phagocytes once attracted to the bacteria accomplish their death, and we have seen that so far Metchnikoff has accepted Ehrlich's explanation of two bodies being concerned in the bactericidal action. The point of difference here is whether this action is entirely extracellular or whether bacteria may be saturated with immune body extracellularly and meet the complement

only intracellularly. But another and an essential aspect of the phagocytic process requires explanation, namely, the facts of positive and negative chemiotaxis,—the attraction which is necessary for recovery from infection, and the indifference or repulsion which accompanies susceptibility. In his earlier work Metchnikoff seemed to look on these phenomena as due to what can only be described as a vital activity of cells. In this connection much harm was done to the theory by the unguarded language used by many of its adherents regarding this manifestation of cellular function. And even yet leucocytes are sometimes spoken of as if they possessed a sentient intelligence. In his latest work Metchnikoff adopts the language of a disciple who remarks that immunisation effects an education of the leucocytes. At the same time, however, he is tending to attempt a physical explanation of the phenomena concerned. Hitherto such an explanation has been applied chiefly to the case of active and passive acquired immunity. It rests on the idea that a bactericidal serum produced by repeated injections of a bacterium contains substances which stimulate the phagocytes to move towards, englobe and digest that bacterium when opportunity occurs. Why this stimulation of phagocytes leads them to move in a particular direction does not transpire. The evidence for the existence of these stimulines as they have been called is as follows. Gengou⁽⁹³⁾ injected mice with the bacillus of swine-fever mixed with its bactericidal serum (heated to 55° C. to destroy any complement) and with the serum of the normal guinea-pig (this contained an adequate complement). The animals did not die. Previous experiments had indicated that when an immune body comes in contact with its appropriate bacilli it becomes fixed to them. Bacilli of swine-fever were now treated with the immune serum and, after the supposed fixation of the immune body, were washed so as to free them of the other constituents of the serum. The experiment just detailed was then repeated, these "sensitized" bacilli being used instead of a mixture of ordinary bacilli and immune serum. The animals died notwithstanding that immune body and complement were present. From this Metchnikoff deduces the presence in the immune serum besides the immune body of some substance stimulating the phagocytes. With regard to this experiment no deduction can be drawn. The details are not given, it rests on the assumption derived from analogy that immune body was sufficiently fixed by the bacilli (no investigation of whether or no immune body was present in the washings of the bacilli is described), and its results are in direct contradiction to

similar experiments performed by Savtchenko⁽⁹⁴⁾. This observer impregnated the red blood corpuscles of the guinea-pig with a haemolytic serum derived from the rabbit and allowing time for the immune body to become fixed he washed away all the other constituents of the serum. These were introduced into the peritoneal cavity of a fresh guinea-pig in which by the previous injection of bouillon an active hyperleucocytosis had been set up. Savtchenko states that when red blood corpuscles (and the contention was borne out here by control experiments) are injected into the peritoneal cavity of an animal of the species from which they were derived, no taking up by leucocytes occurs even when hyperleucocytosis has been caused. He found however that the corpuscles sensitised as described, were quickly taken up, in other words a negative chemiotaxis had been changed into a positive.

These results find support in the experiments of Mesnil⁽⁹⁵⁾ on the bacillus of swine-fever though Metchnikoff quotes the latter in support of his own view. According to Mesnil the effect of injecting immune serum before infection with the bacilli is that these are quickly taken up and digested by phagocytes, both mononuclear and polynuclear. This phenomenon does not happen in the case of an ordinary inoculation followed by a fatal result. That the effect here was due to stimulation of the phagocytes and not to a direct effect of the serum on the bacteria was deduced from the observation that the immune serum *in vitro* had no bactericidal action. This statement however is only partially true. There is no doubt that when an immune serum is quite fresh it has bactericidal properties in many cases, if not all (as Bordet long ago pointed out). When it is kept the delicate complement disappears and old sera have no bactericidal properties, but these can be revived by the addition of fresh complement in the manner already so often alluded to. Thus all that may have happened here may have been that within the animal's body the immune body may have found an efficient complement,—which circumstance gave rise to the death of the bacteria without the immune serum having had any direct chemiotactic action whatever on the phagocytes. While, however chemiotaxis in immune animals might be explicable on the supposition of a stimulation of phagocytes by substances developed in the serum during the process of immunisation, the question of the occurrence of chemiotaxis in naturally immune animals presents a difficulty on this hypothesis. Metchnikoff⁽⁹⁷⁾ drew attention to the fact that the sera of some men could protect the guinea-pig against peritoneal infection with the cholera vibrio. He attributes this to the existence of stimulines in

ordinary sera as well as in immune sera. In the former case they act alone in giving rise to phagocytosis, in the latter their action is reinforced by the immune bodies and also perhaps by the agglutinines. On the other hand, according to Savtchenko's results already quoted, the stimulation of the phagocytes is due to the impregnation of bacteria by the immune body of the immune serum. This view has the merit of being definite. What bodies precisely Metchnikoff refers to under the name of stimulines is very difficult to make out, but probably he considers that the cytases to which allusion has already been made possess this stimulating function in addition to their other powers. Whether when the cytases act alone (as he supposes is the case with normal sera which possess bactericidal action) a different group of these ferments acts from what is involved when their action is reinforced by immune sera does not transpire, but from Metchnikoff's adopting Bordet's view of the singleness of complement one is inclined to the belief that he holds this existence of only one set of ferments within the phagocytes. The fact that he does not believe in the extracellular existence of cytases in any case would lead to the idea that in natural immunity a phagocyte is stimulated to move in a particular direction by something inside its own protoplasm.

Taking all the facts into consideration it must be held that the evidence for the existence of a separate group of bodies having the particular effect of stimulating phagocytosis is of a very nebulous character. At present the observations on the subject are so closely connected with observations on the bactericidal effect which may follow phagocytosis that it is at present difficult to differentiate between a stimulating effect on phagocytes and a bactericidal action. We shall allude later to a certain aspect of the question which arises out of Ehrlich's theory. Meantime it may be remarked that the process may not depend on a chemical but on a physical stimulus. Jennings⁽⁹⁸⁾ for instance has shown that in certain cases chemiotaxis can be influenced by the passage of electrical currents.

The process of which phagocytosis is a part. There is one aspect of phagocytosis which has not received Metchnikoff's attention, and this may best be approached by consideration of what process actually can underlie the so-called education of the leucocytes. Take the case of an immunization against peritoneal infection with cholera. A few bacteria are introduced and the animal does not suffer from a fatal illness. Next a larger dose of bacteria is introduced with the same result. The tolerance of this larger dose (which in the first instance

might have been fatal) is due to the leucocytes having acquired greater phagocytic power. Now is this greater bactericidal power possessed by all the leucocytes of the animal's body or only by those leucocytes which exercised phagocytic action on the bacteria previously injected? It is not outside the regions of possibility that it might only be the leucocytes previously involved which acted on the second occasion. The sensitiveness of protoplasm is of a very exquisite kind, for example in many species of butterfly a male will become aware of the presence of a female though the latter be hundreds of yards away and out of sight. And similarly sensitized leucocytes might be so powerfully attracted by bacteria of a species they had formerly englobed as to pass from distant regions of the body to which in the interval between injections they might have been transported. But here another consideration comes into notice. We know little of the duration of the life of a leucocyte before it is broken up, but it is likely that this period may be measured only by days. On what then does the immunisation process exercise its lasting effect?

The hyperleucocytosis which occurs in many infectious diseases in man often rapidly subsides, and the swelling of the spleen which often occurs at the same time may be an indication of over-activity in that great organ of leucocytic destruction. An active immunisation against infection may persist for long periods of time, but the relation of hyperleucocytosis to such immunisation and to the development of a bactericidal serum and especially the relation, if any, of the subsidence of leucocytosis and of the subsidence of the further accompanying phenomena presently to be alluded to, has not hitherto been the subject of sufficient investigation. In fact the aggregation of phagocytes locally at the seat of infection has drawn attention away from the part played by the phagocyte producing tissues. Within recent years these have been studied by Roger⁽⁹⁹⁾ in France, and independently by Muir⁽¹⁰⁰⁾ in this country. The fact has long been known that in many infectious conditions the number of leucocytes in the circulating blood is increased, but it was left to these observers to demonstrate the extremely pronounced germinative activity which occurs in any severe infection in the precursors of these cells. With regard to the leucocytic phagocytes Muir has shown both experimentally in animals and by observations on man that in infections where there is a polymorphonuclear hyperleucocytosis not only is there evidence of active division of the parent cells in the bone-marrow, but so active is this process that the red marrow increases in amount and encroaches on the yellow. In a case

of pneumonia for instance a few days after the commencement of the disease the red marrow may have increased so as to occupy a seventh part of the whole medullary cavity of the femur. Not only, however, does proliferation occur in the site of formation of such an important class of cells as the polymorphonucleate leucocyte but Muir has also shown that proliferation occurs during some infections in such fixed cells as those lining the sinuses of lymphatic glands and also in the hyaline cells lying free in the lymph sinuses, which latter may be connected with some at least of the large mononucleate hyaline cells of the blood. He further points out that similar hyaline cells,—endothelial cells, connective tissue cells,—proliferate during infection, as can be shown from mitotic figures being found. It is no doubt the case that in different infections different groups of cells thus proliferate; in typhoid fever for instance there is no polymorphonucleate reaction, but here the proliferation of endothelial cells and hyaline cells in lymphatic glands has been observed. Thus while Metchnikoff has insisted with justice on the importance of the local reaction and of the wandering cells of the body in infection, and has noted the occurrence of phagocytosis in other cells (his “fixed amoeboid cells”) he has missed the fact of the great proliferative changes in various parts of the body which may be described as the reaction of the body generally against infection. It must be insisted that there are not only local chemiotactic effects, but in the case of the wandering cells there is the general chemiotactic effect which draws the polymorphonucleate leucocytes from the marrow, and in all cases of severe infection there is the further stimulative effect which leads cells in various parts to divide. Either this stimulation is part of a reparative process or it is to be looked on as the result of injury due, say, to circulating poisons. The fact that, in relation to one aspect of the process, namely, the polymorphonucleate reaction, the effect often is to increase at a given point the available number of cells capable of acting as phagocytes, leads us to think that all these tissue changes may be of the nature of an exaggeration of normal functions, the general effect of which exaggeration is to have a beneficial effect.

It is to be noted as a very important point in this process that most of the distant effects must be due, even in the case of bacteria which *in vitro* do not secrete soluble poisons, to the circulation of soluble toxins unless, which is possible, we consider bacteria capable of emitting purely physical influences. Connected with these is the other very important fact that embryonic activity may be dissociated

from any actual phagocytosis on the part of the proliferating cells, and this taken along with such facts as the proliferation in certain infections of the non-phagocytic eosinophile leucocytes raises anew the question of the possible secretion of chemical substances into the serum which may be concerned in the complicated process by which bacteria are destroyed within the animal body. Here it may be observed that Muir has noted an increase in size and distinctness of the granules in the young polymorphs which occur in the marrow during a severe infection. This might indicate the preparation of material to be secreted. From what has been said *it is thus possible that on the fixed cells of the body and the fixed precursors of the wandering cells are impressed qualities which perpetuate immunity in an animal which has survived an infection.*

These observations and deductions of Roger and Muir open up quite a new field of enquiry, the exploration of which must throw most important light on the whole question of immunity.

(To be continued.)

POST-SCARLATINAL DIPHTHERIA.

(One Figure.)

By W. T. GORDON PUGH, M.D., B.S. (LOND.),

*Senior Assistant Medical Officer, North-Eastern Hospital, Metropolitan Asylums Board, London.*I. *The Statistics of Post-scarlatinal Diphtheria.*

FOR several years returns have been made by the hospitals of the Metropolitan Asylums Board on the subject of this complication, and these are here briefly analysed.

The period under observation may conveniently be divided into two parts, the first including the years prior to the introduction of the bacteriological method of diagnosis and to the use of antitoxin in these hospitals, the second the years subsequent. Previous to 1895 only cases of scarlet fever which showed clinical diphtheria, having membrane in the fauces or exhibiting laryngeal symptoms, were designated post-scarlatinal diphtheria; since that year all cases of secondary throat illness associated with the diphtheria bacillus have been returned as diphtherial, including those which would from the clinical appearance alone have been regarded as simple tonsillitis. It is probable that the majority of the cases which are now seen in these hospitals would formerly have been described as tonsillitis. The significance of this is shown in the fact that only 11·9 per cent. of the cases recorded in the years 1896-1900 presented laryngeal symptoms, whereas in six years belonging to the former period, 1889-1894, laryngeal diphtheria formed no fewer than 52·8 per cent. of the cases occurring at those hospitals which made a return of the character of the cases. It will thus be seen that the first half of each of the two tables that follow is to be regarded as on a totally different footing from the second.

It was, therefore, natural that in the year 1895 there should appear a sudden and large increase in the incidence of secondary diphtheria

among the patients in the scarlet fever wards of these hospitals. It would seem probable, however, in view of the early recognition of

TABLE I.

	1891	1892	1893	1894	1895	1896	1897	1898	1899	1900
Scarlet Fever cases } completed	5,444	11,326	14,867	12,637	10,422	15,054	15,250	12,771	13,327	10,749
Cases of post-scarlatinal diphtheria }	99	217	207	210	453	705	796	661	692	405
Percentage incidence }	1·8	1·9	1·3	1·6	4·3	4·6	5·2	5·1	5·1	3·7

atypical cases of diphtheria by bacteriological means and their consequent prompt isolation, that, in spite of the figures, the number of patients actually infected with this disease has in reality very considerably diminished in recent years.

The influence of the change in the basis of diagnosis and the introduction of a new method of treatment is especially noticeable in Table II., which shows the deaths among patients who have suffered from post-scarlatinal diphtheria. It must, however, be pointed out that

TABLE II.

	1891	1892	1893	1894	1895	1896	1897	1898	1899	1900
Cases of post-scarlatinal diphtheria }	99	217	207	210	453	705	796	661	692	405
Deaths among these ...	55	95	120	77	67	36	30	24	25	12

these are deaths among patients who have died at any period during their stay in hospital subsequent to an attack of secondary diphtheria, and are not necessarily deaths due to this complication. Thus, in the year 1901, when special attention was paid to this point, of 23 deaths following an attack of post-scarlatinal diphtheria, 12 are stated to be from causes unconnected with the diphtheria. The excess of deaths recorded in the table over the deaths really to be attributed to the complication was probably not a very material one while the diagnosis was made on clinical grounds; since 1895, it is obvious, the influence of this system of registration has become much more marked.

For these several reasons it is impossible to arrive at the mortality with correctness. The case death-rate, and even that calculated on the

number of scarlet fever patients treated, are both largely in excess of the true figures.

With the terrible mortality among patients attacked by this complication in the years before the introduction of antitoxin—when diphtheria assisted in producing the fatal issue in over 12 per cent. of the scarlet fever deaths—there may be happily contrasted the results obtained at this hospital in 1901, when of 3,094 scarlet fever patients under treatment only one died who had suffered from secondary diphtheria, even this death being attributable to lobar pneumonia occurring a month after the attack. There are, perhaps, few examples of advance in medical science so striking as the extraordinary reduction in mortality from post-scarlatinal diphtheria.

Sex distribution.—The number of female patients under treatment for scarlet fever, during the five years of which complete statistics are available, was not greatly in excess of the males,—32,395 males; 34,458 females. It will be seen, however, in Table III. that the number of

TABLE III. *Sex distribution in the five years, 1896—1900.*

	All cases		Faucial and Nasal		Laryngeal	
	Males	Females	Males	Females	Males	Females
Cases of post-scarlatinal diph- theria }	1,520	1,739	1,297	1,571	223	168
Deaths among these ...	65	62	36	44	29	18

females developing secondary diphtheria was, proportionately, considerably larger. This increase agrees with the sex distribution among patients suffering from ordinary diphtheria, the number admitted during the same five years being 15,437 males; 17,856 females. The large percentage of males among those with laryngeal symptoms is striking.

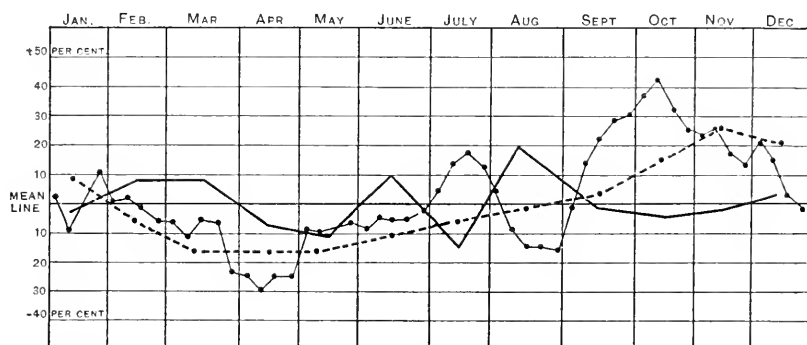
Age distribution.—In connection with this point it should be mentioned that children under three years of age are not usually transferred to the convalescent hospitals, in which the incidence of this complication is considerably higher than at the acute hospitals.

The greatest attack rate is in the fifth year; in the diphtheria notifications in London, per 1,000 living, also, this age is pre-eminent. An age-period curve would follow the same general course as the curve for diphtheria notifications, but the rise and fall would be much more gradual and the curve flattened out.

TABLE IV. *Age distribution in the five years, 1896—1900.*

	under 1	1—2	2—3	3—4	4—5	5—10	10—15	15—20	over 20
Cases of post-scarla- tinal diphtheria	20	75	182	392	471	1,541	452	89	37
Deaths among these ...	7	19	17	27	22	31	4	—	—
Total scarlet fever admissions	634	2,274	4,417	6,314	6,958	26,740	12,080	3,891	3,545
Percent. incidence of post-scarl. diphtheria	3.1	3.2	4.1	6.2	6.7	5.7	3.7	2.3	1.0

Seasonal influence.—If the number of cases of post-scarlatinal diphtheria, developing in each month in the five years under consideration, be averaged, and corrected for the mean daily number of scarlet fever patients under treatment in each of these months, it will be



Dotted line indicates average daily number of scarlet fever patients under treatment.
 Thick line indicates mean percentage incidence of post-scarlatinal diphtheria, calculated on the average daily number of scarlet fever patients under treatment.
 Thin line indicates the weekly number of diphtheria notifications in London.
 The mean line represents 2,721, 1.99 and 1,178, respectively. The chart deals with the five years, 1896—1900.

found that the incidence of this complication does not follow the seasonal variation of diphtheria in the Metropolis, nor does it appear to depend on whether the hospitals are full or the reverse. These points are set forth in the accompanying chart. It must be noticed, however, that the statistics deal only with 5 years and 3,259 cases of post-scarlatinal diphtheria, numbers, perhaps, not sufficiently large to allow of accurate deduction.

Incidence at acute and convalescent hospitals.—In calculating the incidence of post-scarlatinal diphtheria on the number of patients discharged and dead an important correction is necessary. Returns made in the years 1899 and 1900 show that the patients treated to recovery or death in the Board's town institutions have been in hospital about 68 days on an average, while patients who have completed their recovery or died at the convalescent hospitals have been, on an average, 31 days at the town hospital and about 48 days at the convalescent institution. If the calculation be made on "patient-days" (on the

TABLE V.

Incidence at Acute and Convalescent Hospitals, 1896—1900.

	Acute Hospitals	Convalescent Hospitals
Scarlet fever cases discharged to their homes or dead ...	33,296	33,855
Cases of post-scarlatinal diphtheria among these	1,510	1,749

"foot-pound" principle), it will be found that the liability to post-scarlatinal diphtheria at the convalescent hospitals is *about two and a third times* as great as at the town hospitals.

II. *The Origin of Post-scarlatinal Diphtheria.*

I now propose to consider the principal reasons which have been suggested for the occurrence of secondary diphtheria in scarlet fever wards.

(i) *Sanitary defects.*—As advances have been made in our knowledge of the bacterial origin of diphtheria, the belief, once generally held, that defective drainage played an important part in disseminating this disease has gradually waned. However, it may be well to recall that Sweeting¹, in 1893, investigated this point in connection with the Board's hospitals, and found that post-scarlatinal diphtheria had prevailed in like degree in hospitals with ventilated and in those with unventilated soil-pipes; in hospitals with automatic flushing apparatus, and in hospitals without such appliances; in hospitals with elaborate systems of ventilation and disconnection, and in hospitals where these were of the most meagre and incomplete kind. In fact, the diversity

¹ *Trans. of Epidem. Soc., Lond., xii.*

was so great that no common factor of drainage defect could be pointed to as explaining the long-continued yearly recurrence of this condition of post-scarlatinal diphtheria.

(ii) *The treatment in the same hospital of the two diseases.*—It is but natural that a layman, unacquainted with the administration of a fever hospital, should, when he hears that his child, convalescent from scarlet fever, has developed diphtheria, forthwith conclude that infection has been derived from cases of diphtheria treated in the same hospital. This opinion has to some extent been shared by members of our own profession. Thus, Sweeting apparently believed that there was a connection between the reception of both diseases in the Board's hospitals and the incidence of post-scarlatinal diphtheria. He concluded from a study of his statistics that "there had been a marked increase of the complication at the acute hospitals since diphtheria was received, although it had undoubtedly existed to a minor extent at some of them before diphtheria was admitted," but "...that at the Northern Convalescent Hospital it had existed before and after the reception of diphtheria convalescents, and that its prevalence had apparently been inappreciably affected thereby."

Now if, as the supporters of this theory have held, the treating in the same hospital of the two diseases is the main cause of post-scarlatinal diphtheria, one would expect it to be of comparatively rare occurrence in hospitals reserved entirely for the treatment of scarlet fever. That this is not so is evident from the fact that 160 cases were recorded for this (the North-Eastern) hospital during the five years, 1896–1900, during which period only patients certified to be suffering from scarlet fever were received. Similarly at Gore Farm, which up to 1899 received scarlet fever convalescents only, 273 cases of secondary diphtheria occurred during the two years 1897 and 1898.

It would be interesting to compare the incidence of post-scarlatinal diphtheria at hospitals receiving scarlet fever only and at those admitting both diseases. In the case of the acute hospitals such comparison would, however, be without value, on account of the varying proportion of patients transferred to the convalescent institutions. Comparison of the latter hospitals is free from this particular objection:—at Gore Farm during the years 1896–98, a period when it received scarlet fever convalescents only, 4·5 per cent. of the patients developed secondary diphtheria; at the Northern Hospital, admitting convalescents from both diseases, the almost identical percentage incidence of 4·9 is recorded during the same three years.

It may, therefore, I think, be regarded as proved, so far as statistics are able to help one, that the aggregation upon the same site of the two diseases is not an important factor in the etiology of post-scarlatinal diphtheria. Is it possible for such association ever to give rise to this complication? Goodall¹ in 1896, after pointing out certain fallacies in Sweeting's statistics, said he had not been able to satisfy himself that, save in very exceptional instances, infection had been conveyed from the diphtheria to the scarlet fever wards. Indeed, since diphtheria spreads solely through intimate contact with the source of infection, it can extend to the scarlet fever wards only in consequence of imperfect separation of the convalescents or through conveyance there by members of the staff. I am not aware that the first means of infection exists at any hospital and will, therefore, confine my remarks to the second.

Practically the only persons involved are the medical officers and the nurses. The former, however, are not brought into sufficiently close contact with their patients to encourage the belief that they serve in any degree of frequency as sources of infection. The intimate relations, on the other hand, existing between a nurse and the children under her care render her more likely to prove an important factor in the spread of this disease. On a later page the liability of a nurse in close attendance on diphtheria patients to acquire virulent bacilli will be pointed out. Is there any evidence that a nurse, in the best of health herself, can by this means convey infection? Proof has before now been furnished, but the following instance seems of sufficient interest to deserve mention.

Prior to the opening of our diphtheria wards there was an isolation building in this hospital, used for cases erroneously diagnosed as scarlet fever, containing four separate rooms, which were looked after by a single nurse. In one was a child with bronchitis; in another a patient suffering from diphtheria. The latter died on November 18th, five days after admission. On December 3rd the bronchitic child, who had not yet left his bed, developed laryngeal diphtheria necessitating tracheotomy. No source of infection appeared possible save by the medical or nursing staff. Cultures were made from the throats of all who had been in contact with the child, and from one nurse, who had been in attendance on the diphtheria case of a fortnight before, virulent Klebs-Loeffler bacilli were obtained. She had throughout had no sore throat, and the tonsils showed only chronic enlargement.

¹ *Trans. of Epidem. Soc., Lond., xv.*

This subject has been dealt with at some length because it appears to be the only conceivable way by which the disease can be conveyed from the diphtheria to the scarlet fever wards.

(iii) *The introduction of unrecognised diphtheria.*—That this is the usual source of infection there can be little doubt. It is interesting to note how the yearly incidence of post-scarlatinal diphtheria in fever hospitals has varied with the prevalence of diphtheria outside. Thus Meredith Richards¹ stated that in 1893, 1894 and the first half of 1895 there were no cases of this complication noted at the Birmingham Fever Hospital, into which diphtheria was not admitted. In July, 1895, fatal laryngeal diphtheria occurred in a convalescent child, and this was followed by secondary cases and numerous other outbreaks during the rest of the year. The diphtheria deaths recorded for Birmingham had been 43 in 1893, and 50 in 1894; in 1895 there was a sudden rise to 163. This agrees with the experience in the hospitals of the Metropolitan Asylums Board, the incidence of post-scarlatinal diphtheria had been 0·2, 0·4 and 0·4 per cent. in 1885–87 respectively. In 1888 it rose to 1·1 per cent. This coincided with a sudden bound in the diphtheria death-rate per million in London, from 235 in 1887, to 319 in 1888. It happened that it was in the latter year that the managers first received diphtheria cases into their hospitals, but, as Goodall² pointed out, the increase in post-scarlatinal diphtheria was noticeable before October 23rd when the diphtheria wards were opened, and only 99 cases of the latter disease were admitted between that date and the end of the year. Thus, the suggestion that the rise was due to the reception of diphtheria patients falls to the ground.

Among the cases received into fever hospitals certified scarlet fever, a few can be readily recognised clinically as uncomplicated diphtheria. A larger class is that in which there is on admission evidence only of tonsillitis, for patients have not infrequently lost by the time they arrive at the hospital the other signs upon which the practitioner founded his diagnosis, and yet many of these are proved subsequently, by the occurrence of desquamation, to be suffering from the disease certified. Owing to the limited number of isolation rooms, a considerable proportion of these cases of apparent tonsillitis are admitted for observation into the scarlet fever wards, and one of mild diphtheria might thus be the origin of an outbreak of post-scarlatinal diphtheria.

¹ *Lancet*, Sept. 26th, 1896, p. 876.

² *loc. cit.*

Another class of case is that of double infection. Occasionally, in addition to the signs of scarlet fever, the patient presents undoubted diphtheritic membrane in the throat. These cases, however, must not be confounded with a much larger number in whom a condition of throat more or less simulating diphtheria is found; cultures, as a rule, show absence of the specific bacillus, but sometimes organisms are found which morphologically are indistinguishable from it. It will be shown later that non-virulent diphtheria bacilli are not uncommon in normal and scarlatinal throats; hence, in the absence of definite membrane, the mere finding of the bacillus, without recourse to inoculation, cannot be regarded, in this throat condition, as proof of the co-existence of the two diseases. However, it cannot be denied that cases of the combined diseases may occasionally be admitted in which the local evidence of diphtheria is so slight, or else so masked by the lesions of scarlet fever, as to escape recognition. Finally, as is well known, there may, under certain circumstances, be present in throats which are apparently quite healthy, virulent bacilli which are capable of causing diphtheria in other patients.

So far I have dealt only with the conveyance of infection in the throat. There is another source of infection which is, I believe, of almost equal importance, the nose. A disease which is known to rhinologists as fibrinous rhinitis, but which I see no logical objection to calling nasal diphtheria, appears to be not at all uncommon among children. Producing, as it does, little or no constitutional disturbance and no external evidence save discharge and soreness—and even these may be almost absent—it is a disease which is easily overlooked.

The whole subject of the presence of diphtheria bacilli in the throat and nasal cavities, and of the significance of these organisms under various conditions, is of such vast importance in dealing not only with post-scarlatinal diphtheria, but also with outbreaks in institutions and towns, that it has been thought well to enter into it in considerable detail in the following sections, in order that the limitations which exist to the usefulness of bacteriology in such endeavours may be the better appreciated.

III. *Diphtheria Bacilli in the Throat.*

An examination of the literature of diphtheria will reveal the fact that considerable lack of uniformity has existed in describing and naming diphtheroid organisms, a fact which depreciates the value of many of the observations. In the following consideration of the

occurrence of diphtheria bacilli under various conditions, I have selected for illustration investigations in which there has apparently been adopted the classification which is now in general use, founded on one suggested by Park and Beebe¹ in 1894, namely, (i) the virulent diphtheria bacillus (Klebs-Loeffler), (ii) the non-virulent diphtheria bacillus, and (iii) the Hoffmann bacillus. It is unnecessary for the purpose of this paper that the methods of distinguishing between the diphtheria bacillus and that of Hoffmann should be entered into in detail, but it may be stated briefly that, to confirm the microscopical differentiation, Neisser's double stain and the reaction produced by growth in neutral litmus glucose broth are, apart from tests of virulence, the chief methods relied on.

By the vast majority of bacteriologists it is believed that only bacilli of the first group are capable of causing diphtheria. By some writers, however, Hoffmann's bacillus is said to cause a mild tonsillitis, but I have never been able to convince myself that, in any of the numerous cases of tonsillitis which have occurred at this hospital, its presence was ever more than accidental. As will be shown later a considerable percentage of children have normally these bacilli in nose or throat. By a few observers the possibility of the conversion of Hoffmann's bacillus into the virulent diphtheria bacillus has been asserted, but the evidence of this cannot be regarded as satisfactory.

(i) *In scarlet fever patients on admission into hospital.*—A series of 420 unselected cases received into this hospital, certified scarlet fever, were examined as to the presence of bacilli in the throat on admission, the cultures being taken in the receiving room to avoid complication. The inoculations were kindly performed by Dr Cartwright Wood.

The cases may be described in relation to clinical diphtheria.

Two were uncomplicated faucial diphtheria. One was easily recognizable as such; the other presented follicular deposit only. A culture from the latter was tested by inoculation and the bacilli were found to be virulent.

One case was scarlet fever associated with a croupy cough and considerable obstruction to respiration. Diphtheria bacilli were obtained from the fauces, which were inflamed but without deposit.

Two were scarlet fever complicated by fibrinous rhinitis, the presence of which was recognized in the receiving room. From the throats of both these patients diphtheria bacilli were obtained, although there was clinically only the inflammation of scarlet fever.

¹ *New York Medical Record*, 29th Sept. 1894.

In 17 of the remaining 415 cases was the diphtheria bacillus found. In three of these the fauces were merely congested; one culture was tested by inoculation and proved non-virulent. Nine showed inflammation without deposit; three of these were examined as to pathogenicity with negative result. Three cases presented follicular exudation. One case showed ulceration of tonsils and uvula, and one case a pultaceous mass of exudate on the tonsils with some ulceration; bacilli from the latter case were found on inoculation to be non-pathogenic to guinea-pigs. Thus, in five of the cases which clinically did not suggest being complicated with diphtheria, the inoculation test was applied with a negative result; the bacilli in each of these cultures stained with Gram's and Neisser's solutions, and rendered neutral litmus glucose broth acid.

Hoffmann's bacillus was found in 67 of the cultures.

Garratt and Washbourn¹ examined the throats of 666 cases of scarlet fever admitted under their care at the London Fever Hospital from March, 1896, to December, 1898. In eight, or 1·2 per cent., were found bacilli morphologically resembling *B. diphtheriae*. The inoculation test was not applied; in only one case was there reason to suspect the presence of diphtheria from the clinical appearance of the throat; in another case there was a history of intimate exposure to diphtheria. It will be noticed that this percentage is considerably lower than that found in the patients admitted into this hospital. The difference is possibly dependent on the higher average age and social status of patients at the London Fever Hospital. The relative frequency among the class of patients admitted to the Board's hospitals is confirmed by an investigation of Goodall's² in 1896, when among 87 cases of scarlet fever examined on admission six patients were found to have diphtheria bacilli of the long variety in their throats.

(ii) *Among the general public.*—It will be interesting now to consider the presence of these organisms among the general public. Apart from investigations in connection with outbreaks and epidemics, however, there are but few reliable accounts of the examination of healthy throats.

Theoretically, one would expect that the prevalence of the diphtheria bacillus would depend on whether diphtheria was endemic or not in the locality, that the proportion of persons involved would vary with the season, and that important factors would be the age of the persons

¹ *Brit. Med. Journ.*, 15th April, 1899, p. 893.

² *loc. cit.*

examined, their social status and consequent surroundings. The results however, in limited researches, such as the following, must be subject to many variations, and no one percentage can be accepted as expressing the actual condition.

Denny¹, of Brookline, Mass., examined 235 healthy individuals, 216 children and 19 adults, a large proportion being of the well-to-do class. In cultures from their throats only once was the diphtheria bacillus found. This was a school-girl, who, as far as was known, had not been in contact with any case of diphtheria. The bacilli were so few that a pure culture for inoculation could not be obtained.

Park and Beebe², of New York, on the other hand, examined 275 persons, chiefly hospital and dispensary patients, who were not known to have been exposed to infection; from the throats of 26 diphtheria bacilli were obtained, which in no fewer than 23 cases proved non-virulent to guinea-pigs. Of these persons 50 were adults, among whom non-virulent bacilli were found twice. One of the three cases with virulent bacilli was found to be from a house where a case of supposed croup had existed three weeks before.

Kober³ examined 600 school-children, whom, he says, he selected from the lowest standards because diphtheria was more common at that age. In fifteen diphtheria bacilli were found, in each case staining by Neisser's method and rendering glucose broth acid; ten, however, proved non-virulent to guinea-pigs. Of the five children with virulent bacilli, one sat at school next a child who had had diphtheria eight weeks before, three were playmates of neighbours' children who had had diphtheria recently, and the fifth had associated with a family in which a fatal case of the same disease had occurred ten weeks before. With regard to the ten children with non-virulent bacilli, in five cases no connection with diphtheria could be traced, in four there had been diphtheria in the same or the next house, while the remaining boy had played with a child who had had this disease some time before.

Hewlett and Murray⁴ examined bacteriologically the throats of all the children (385) received into the Victoria Hospital for Children, London, for diseases other than diphtheria during 1900. In no fewer than 58 (15 per cent.) the Klebs-Loeffler bacillus is stated to have been found, in 92 (24 per cent.) the bacillus of Hoffmann. Seven of the 58

¹ *Boston Med. and Surg. Journ.*, 22nd Nov., 1900, p. 516.

² *Amer. Journ. of Med. Sciences*, Oct. 1894.

³ *Zeitschr. f. Hygiene*, 1899, Bd. xxxi., s. 433.

⁴ *Brit. Med. Journ.*, 15th June, 1901, p. 1474.

presented some clinical evidence of diphtheria; in three of these, however, it is remarked the bacillus was not found at the first examination of their throats, but they were sent to a fever hospital 5, 18, and 23 days' respectively, after admission, diphtheria bacilli being then present. Three of the cultures were tested as to virulence, but it is not related in the paper from which class of case they were obtained. Two were not lethal to guinea-pigs; the third proved virulent.

Similarly, Steenmeyer¹ says that, in Rotterdam, he found in 7 per cent. of persons examined diphtheria bacilli in the normal mucous membrane of the pharynx.

(iii) *Among nurses in attendance on diphtheria patients.*—The wards for the treatment of diphtheria in this hospital are new, well-ventilated and well-lighted, and the cubic space per patient is unusually large. The nurses have been instructed as to the dangers attached to the acquiring of diphtheria bacilli in the throat, and it is to be presumed are careful to avoid unnecessary risks. Nevertheless, in a series of single cultures made from the throats of 56 nurses working in these wards diphtheria bacilli were found in seven. One of these cultures was submitted to the inoculation test and proved virulent to the guinea-pig. The throats of all were clinically normal. In scarlet fever wards nurses appear to be less particular and more given to the fondling of children; and in cultures made in connection with cases of post-scarlatinal diphtheria a larger proportion of nurses have sometimes been found to harbour the bacillus. It is interesting to observe, however, that bacilli found even under these circumstances are not necessarily virulent. For instance, it happened that several cases of tonsillitis occurred in one of our convalescent scarlet fever wards. Cultures showed a rather short diphtheria bacillus, and similar bacilli were obtained from a few of the other patients with normal throats. Cultures were made from the nurses. One was found to have the same short bacillus in her throat; another had very long diphtheria bacilli of quite another type. The former developed a small patch of membrane a day or two later. It being suspected that the long bacilli of the second nurse were not connected with the outbreak, their virulence was tested;—although the bacilli showed well-marked pole granules with Neisser's reagents, and produced acid fermentation of glucose broth, a negative result followed the inoculation of a guinea-pig.

(iv) *Among others who have been exposed to infection.*—When cultures are made from convalescent patients occupying a scarlet

¹ "Dissertation," Utrecht. Ref. *Baumgarten's Jahresbericht*, 1898, s. 216.

fever ward in which a case of secondary diphtheria has occurred, it is usual to find *B. diphtheriae* in some of the normal throats. In several cases at this hospital these cultures have been tested by inoculation, and found to be virulent.

Many other investigations might be quoted proving the liability of those who are brought into close contact with patients suffering from diphtheria to acquire virulent bacilli in their throats without showing any signs of the disease.

Johannessen¹ found the virulent bacillus present in the healthy throats of three out of 20 children in a ward in which a case of diphtheria had occurred.

Park and Beebe² examined the throats of the healthy children of 14 families in which one or more of the other members had diphtheria. There were in all 48 healthy children; in 13 of the families and in 50 per cent. of the children diphtheria bacilli were found. Six cultures were tested for virulence with positive results.

Kober³ examined cultures taken, by the doctors in attendance, from the throats of 128 persons who were in contact with people ill with diphtheria. In 15 he found diphtheria bacilli, which in each case proved virulent. In 10 of the cases the throat was normal; in 5 there was slight angina.

(v) *In institutions*.—Goadby⁴ in 1898 examined bacteriologically the throats of 100 healthy children in an industrial school where no diphtheria had occurred for two years. Carbol-methylene blue and Neisser's stain were used, and Hoffmann's bacillus differentiated. Diphtheria bacilli were found in 18 of the cultures. Whether these were of the second group, the saprophytic or non-virulent variety, was not ascertained, the inoculation test, as Mr Goadby has kindly informed me, not being applied. I might mention, however, that in the throat of a child admitted to the North-Eastern Hospital for scarlet fever from a large orphanage, in which no case of diphtheria had occurred for two years, acid-forming bacilli, indistinguishable from the *B. diphtheriae*, were obtained, which on inoculation were found to be non-pathogenic to the guinea-pig.

Goadby was at the time investigating an epidemic of diphtheria at the Poplar Union Schools, where about 600 children are kept on barrack principles, there being but one play-room for each sex.

¹ *Deutsche med. Woch.*, 1895, xxi.

² *New York Medical Record*, 29th Sept., 1894.

³ *loc. cit.*

⁴ *Trans. of Epidem. Soc.*, Lond., xix.

Twenty-three cases of diphtheria had already occurred when the cultures were taken. No fewer than 190 (32 per cent.) out of the 586 children examined were found to have diphtheria bacilli in their throats. What proportion of these were of the virulent variety it is impossible to say; cultures from two children, who had no clinical signs of throat affection, were found to be fully virulent. Only 15 of the 190 subsequently developed clinical diphtheria.

Park and Beebe¹ examined 55 children in a foundling hospital, where from time to time cases of true diphtheria had occurred. Among them six were found to have diphtheria bacilli, five of the cultures being of the virulent variety.

Aaser², in an outbreak of diphtheria in a soldiers' barracks, found the bacillus in 17 out of 89 healthy throats. Denny³ in 1899 examined the throats of 200 boys in a truant-school, in which four cases of diphtheria with membrane had occurred. In 22 the cultures gave a positive result; only six of these boys had sore throats, the others being apparently quite healthy. Berry and Washbourn⁴ met with like results in an examination, under similar circumstances, of the throats of children at the London Orphan Asylum in 1898. In none of these investigations, however, is it stated that the virulence of the bacilli was examined.

(vi) *In epidemics of diphtheria.*—Cobbett⁵, in connection with an outbreak of diphtheria occurring among children attending several of the day-schools at Cambridge in the autumn of 1900, examined 650 persons not suffering from diphtheria, who had been directly or remotely exposed to infection, about 300 being children attending the schools or brothers and sisters of these pupils. Nineteen were found to harbour diphtheria bacilli; a few of these had slight sore-throat at the time of examination. In 8 the inoculation test was applied; 5 were found to be virulent, and 3 non-virulent.

In the Spring of 1901 there was a recrudescence of the disease at Cambridge and the investigation was resumed. Eighty-four children, attending three schools where there had been 2, 1 and 0 cases, respectively, of diphtheria, were cultured with negative result. Sixty-three boys at another school with 160 scholars, where several cases of diphtheria had occurred, were examined, with the result that diphtheria bacilli were found in 10; 7 of the cultures were tested by inoculation,—3 proved virulent, 4 non-virulent.

¹ *loc. cit.*

² *Deutsche med. Woch.*, 1895.

³ *loc. cit.*

⁴ *Trans. of Epidem. Soc.*, Lond., xix.

⁵ *Journal of Hygiene*, i., pp. 228, 235, and 485.

Graham-Smith¹, dealing with a similar outbreak at Colchester, cultured the healthy throats of children living in homes in which a case of diphtheria had occurred within from 3 to 4 months of the examination; 407 scholars from 19 schools, 59 persons above or below school age, and 55 persons from the Colchester Union,—519 in all—were examined. In 54, or 10·4 per cent., were Klebs-Loeffler bacilli found in the throat.

In the preceding paragraphs an attempt has been made to present the circumstances and results of various investigations succinctly, but without omitting points of importance in connection with the subject under consideration. It will be interesting now to consider the significance of these bacilli. As has already been said, it is almost universally held that only those diphtheria bacilli which are pathogenic to the guinea-pig are capable of causing the disease in man. Can the virulent bacillus lose its virulence in nature? Does the non-virulent bacillus under any circumstances become virulent?

Artificially, Roux and Yersin found it was possible to produce an attenuation of the virulence of the bacillus in a number of ways². For instance, if a current of sterile air was kept passing through a broth culture maintained at a temperature of 39·5° C., after about two weeks some of the bacilli began to lose their virulence, and at the end of about four weeks all the bacilli had lost their virulence and produced non-virulent cultures; a little while after losing their virulence the bacilli remaining in the culture died. It has not yet been directly proved that such a change occurs naturally. Park³, in 1900, stated that in his experience bacilli, which at the beginning were virulent, continued virulent so long as they remained in the throat. Cobbett⁴ says that in seven cases where the diphtheria bacilli present in the throat were tested on from 2 to 10 occasions the virulence was found constant. It is interesting to note, on the other hand, that the non-virulent bacillus was obtained in a culture taken from a patient examined for the first time during convalescence from diphtheria. The number of experiments recorded, however, is but small, and the point can hardly be regarded as settled. That such a change does occur naturally is evident from the results of inoculation in the series of observations summarized above. Roux and Yersin found that if from time to time cultures were made

¹ *Journal of Hygiene*, II., p. 170.

² Ref. Park and Beebe, *loc. cit.*

³ *Trans. of the Assoc. of Amer. Physicians*, xv., 1900, p. 222.

⁴ *loc. cit.*

from dried bits of membrane, a period finally came when the bacilli, although alive, had become non-virulent. It is possible that this is one way in which the natural transformation of the bacillus occurs; as may be seen in the above investigations when the bacillus is acquired by infection from a patient suffering from diphtheria it is usually found to be virulent, whereas when the source of infection is remote or untraceable the bacillus is generally of the non-virulent variety. It may be added that it seems extremely probable that the non-virulent bacillus can be, as such, transmitted from one child to another.

The second question must, in the present state of our knowledge, be answered in the negative. Roux and Yersin were unable to give back virulence to those bacilli which had been completely robbed of it by the above method, or to those which had no virulence originally when obtained from the throat. Their attempts were more successful when they used a bacillus that still retained some slight action on the guinea-pig—by injecting a mixture of this non-fatal bacillus and very active cultures of the streptococcus of erysipelas, virulence was, though not invariably, restored. Shattock¹ cultivated these bacilli in broth over which faecal air was passed, but no toxic properties were acquired even though the treatment was prolonged for a period of two months. Lubowski² found he could not render the non-virulent bacillus pathogenic to guinea-pigs by repeatedly passing it through these animals. Cobbett³, recovering the non-pathogenic bacilli from the tiny abscess produced by their inoculation, also tried the effect of passage through guinea-pigs—once four animals in succession—with negative result.

Returning now to the question of the significance of the diphtheria bacillus in the throats of scarlet fever patients on admission to hospital, and regarding it in the light of these investigations, we must, I think, conclude that in large centres of population, where diphtheria always exists, these organisms are to be found in a not inconsiderable proportion of school-children; that, in the absence of evidence of clinical diphtheria and of a history of exposure to that affection, the bacilli are, however, in the majority of cases of the non-virulent or saprophytic type and, therefore, of little hygienic importance; that in cases, on the other hand, where the clinical supports the bacteriological examination the bacilli are almost certainly virulent, and therefore dangerous; while in cases where the patient is known to have been

¹ *Path. Soc. of Lond. Trans.*, 1898.

² *Zeitschr. f. Hygiene*, Bd. xxxv., p. 87.

³ *Journal of Hygiene*, 1., p. 497.

exposed to infection the chances are great that the organisms are of the pathogenic variety, and such cases should always be regarded with grave suspicion.

IV. *Diphtheria Bacilli in the Nasal Cavities.*

Apart from the subject of fibrinous rhinitis, the literature in relation to the presence of diphtheria bacilli in the nose is by no means extensive.

(i) *Among scarlet fever patients on admission into hospital.*—From the noses of 420 patients admitted into this hospital certified scarlet fever, cultures were made with a view to ascertaining the prevalence of bacilli morphologically resembling the diphtheria bacillus. Owing to the luxuriant growth of other organisms found in the noses of scarlet fever patients, the character of the culture often did not admit of the satisfactory examination of single colonies, while the frequent presence of Hoffman's bacillus considerably increased the difficulty.

Clinically, two patients were suffering from faucial diphtheria without evidence of scarlet fever; diphtheria bacilli were found in the throats of both, but in the nose of one only.

One patient had scarlet fever complicated with laryngeal diphtheria: the Klebs-Loeffler bacillus was obtained from both throat and nose.

In two cases of scarlet fever, examination of the nasal cavities in the receiving room revealed the presence of well-defined membrane, limited to the Schneiderian lining and adherent to septum and inferior turbinated bones. Diphtheria bacilli were obtained from throat and nose.

One child came with the history that her two sisters were in the hospital suffering from diphtheria. Cultures from the throat were negative, but diphtheria bacilli were obtained from the nose, although the nasal cavities appeared quite normal. The bacilli were found to kill guinea-pigs in 48 hours in the usual dose of broth culture; when antitoxin was simultaneously injected the guinea-pig remained unaffected.

The remaining 414 cases presented on careful inspection no evidence of either faucial diphtheria or fibrinous rhinitis; nevertheless, from the nasal cavities of 33 were obtained bacilli, morphologically indistinguishable from the Klebs-Loeffler bacillus. In 10, the organisms were found in both throat and nose; bacilli from the noses of two of these, and from the throat of another, were tested and found to be non-virulent. In 23, the bacilli were present in the nose only; three of these cultures were inoculated into guinea-pigs and similarly found to be non-virulent.

Thus, in the six cases in which the pathogenicity was examined, the bacilli, although capable of producing an acid reaction in glucose broth, were found to be non-virulent to guinea-pigs.

In 234 cases, Hoffmann's bacillus was present in the nose cultures. In about thirty of these, all very mixed cultures, there were found in the films spread a few short, straight, rather thick bacilli, with uniform diameter and rounded ends, which presented fair-sized pole granules both when treated with Loeffler's stain and that of Neisser. These rods were sparsely scattered among the Hoffmann bacilli, sometimes singly but generally in small groups. Pure subcultures could not be obtained, so that it was not determined whether they were to be regarded as a variant of Hoffmann's bacillus exhibiting pole granules, or as a short type of the diphtheria organism intimately mixed with these bacilli in the specimen. The treating of them as being of no more serious import than Hoffmann's bacillus has not been followed by ill-result, but their occasional presence adds considerably to the labour of a routine bacteriological examination of the nose.

(ii) *Among the general public.*—Mr Lambert Lack¹ made cultures from the noses of all the children attending his ear, nose, and throat out-patient practice during the first fortnight in October, and 25 children under 12 years of age attending a medical clinique—in all, 100 patients. About 40 were cases of adenoids, four had atrophic rhinitis, many had slight running from the nose, while none were seriously ill, and in no case was there a history of exposure to diphtheritic infection. In 13 per cent. the diphtheria bacillus was found, and in 52 per cent. that of Hoffmann. Unfortunately, as Mr Lack informs me, in no case was the virulence tested.

The statistics of Gross² at the Children's Hospital at Boston, while not to be regarded as indicating the prevalence of diphtheria bacilli in children when admitted into hospital, are interesting because they confirm the above experiences as to the greater frequency of these organisms in the nose than the throat. All the cases entering the hospital during 6 months—316 in all—had culture examinations made of throat and nose, two cultures, 24 hours apart, being taken on admission and subsequently repeated once weekly as long as the cases remained in the house. Twenty-six, at one time or another, showed Klebs-Loeffler bacilli; two only of these had clinical diphtheria. Of the 314 children without clinical diphtheria, 7 had the bacillus in the throat and 17 in the nose, during some period of their stay in hospital.

¹ *Med. Chir. Trans.*, LXXXII.

² *University Medical Magazine*, Oct., 1896.

(iii) *Among nurses in attendance on diphtheria patients.*—Cultures were made from the noses of 25 nurses working in our diphtheria wards; in two were found bacilli indistinguishable from the Klebs-Loeffler bacillus. The nasal cavities in each appeared normal.

(iv) *In post-scarlatinal rhinorrhoea.*—Chronic rhinitis with sore nostrils, a varying amount of discharge and a tendency to the formation of pustules on parts of the body, is a fairly common sequel of scarlet fever. Todd¹ in 1898 called attention to the fact that this "external rhinitis" appeared to be sometimes due to the Klebs-Loeffler bacillus. Fifty-one cases associated with this bacillus occurred among 365 children under observation during 18 months at the London Fever Hospital. The affection, which appeared to be contagious, was most common at the ages of 3 and 4 years, no case occurring after 12; the nostrils were excoriated and crusted; discharge was usually slight and not uncommonly absent. In only three cases were diphtheria bacilli found in the fauces of the children affected; of these two were sisters, whose mother and brother were suffering from definite diphtheria, while the third had been similarly exposed to infection. During the 18 months over which these observations extended a bacteriological examination of the fauces of every patient was made before admission to the scarlet fever wards, and during this period only one case of post-scarlatinal diphtheria occurred in the hospital. In a few instances the virulence of the bacilli was tested, and they were found to be pathogenic to guinea-pigs.

It is not made apparent in the paper that the condition of the Schneiderian membrane was examined in these patients. In similar cases at the North-Eastern Hospital it is not uncommon to find that the condition is, in reality, fibrinous rhinitis. Since the exudation does not extend into the vestibule it may be overlooked unless a mirror be used to inspect the nasal cavities, while a previous syringing is often necessary in order that the view may not be obstructed by mucus. A few details of cases in which the inoculation test has been applied may be given.

A case of secondary diphtheria with membrane on the tonsils having occurred in a convalescent ward, the throats and noses of all the patients were examined. One boy, who had very slight discharge and no redness or soreness of nostril, was found to have the left side of the septum markedly congested with thin but definite membrane extending

¹ *Lancet*, 8th May, 1898, p. 1458.

over part of its surface. Free bleeding occurred when a portion of the membrane was removed, and cultures showed that virulent bacilli were present. The right nasal cavity appeared normal; the throat, also, was natural and a culture from it negative. From two other cases, occurring in different wards under somewhat similar circumstances, bacilli, pathogenic to the guinea-pig and capable of neutralization by antitoxin, were obtained; these patients had rhinorrhoea with scabbiness of the nostrils, and when the nasal cavities were examined after syringing membrane was seen adhering to the turbinated bones. In all the general health remained practically unaffected, and the patients recovered without showing signs of paralysis or any throat lesion.

Yet another outbreak may be mentioned. In a convalescent scarlet fever ward, occupied chiefly by girls of five, a child who developed rhinorrhoea was found to have membrane on the septum and the roof of the bony orifice of each nasal cavity; on the left tonsil was a small area of deposit. Cultures showed the presence of diphtheria bacilli in throat and nose. The bacilli from one nostril were found to produce a strongly acid reaction in sugar broth, and 2 c.c. of a 48-hour broth culture proved lethal to a guinea-pig at the end of the second day, this result being prevented in another guinea-pig by simultaneous injection of antitoxin. In three rounds of cultures made from the other 25 patients, who were kept in bed meanwhile, no fewer than ten were found to have acquired the diphtheria bacillus. The spread of infection appeared to have been assisted by the fact that among the toys of the ward were school-slates which were used indiscriminately by all. Well-marked fibrinous rhinitis was present in two; their throats were normal, although in one diphtheria bacilli were present there also. The other eight children had diphtheria bacilli in the throat, which presented no abnormality except in one case, where there was a thin sheet of membrane on the right tonsil. In two the organisms were present in the nasal cavities also; these clinically appeared normal. Bacilli from each of the cases of fibrinous rhinitis, and from one of the healthy throats, were found to render glucose broth acid, and 48-hour broth cultures in 2 c.c. doses proved fatal to guinea-pigs within 48 hours, while in each case this effect was prevented in control animals by antitoxin.

In dealing with cases of rhinorrhoea which are not accompanied by evidence of fibrinous rhinitis, although associated with the presence of diphtheria bacilli in the discharge, it must not be forgotten that a scarlet fever patient who is the host of non-virulent diphtheria bacilli

may, of course, develop the ordinary post-scarlatinal rhinorrhoea. Hence, isolated cases occurring in wards may well be of this nature. This view is borne out in an examination made by Williams¹ of cases of rhinorrhoea in the scarlet fever wards of the Northern Convalescent Hospital. Unfortunately, the condition of the Schneiderian membrane was not noted in the paper. In 57 out of 141 cases of rhinorrhoea were found organisms which resembled either the Hoffmann or the diphtheria bacillus; in 36 of these the bacilli were present on reception into the Convalescent Hospital. Certain of the cultures were submitted to inoculation; of the 17 strains examined, 4 were acid-producing and virulent, 8 were acid-producing but non-virulent, while 5 were Hoffmann's bacillus.

(v) *Fibrinous rhinitis under other conditions*.—Lambert Lack² found among 700 new cases attending, in one year, his clinique for ear, nose, and throat affections at the Children's Hospital, Paddington Green, 16 cases of this disease, nasal obstruction being usually the symptom for which relief was sought. During the period of his investigation Mr Lack had under his care, altogether, 36 cases; of these, 33 were under 8 years of age, while half the cases occurred during August and September. Thirty-three cases were examined bacteriologically, and in each diphtheria bacilli were found; 23 cultures were tested for virulence with positive result.

Although this affection is more common in young children it is not confined to them. Recently a woman, aged 27, suffering from scarlet fever, was admitted into this hospital with well-marked fibrinous rhinitis; diphtheria bacilli were found in cultures from the nose, and casts of the nasal fossae were subsequently syringed away.

I might add that I have, on several occasions, seen fibrinous rhinitis develop in patients convalescent from ordinary diphtheria some weeks after the throat was clear.

The futility of attempting to stamp out infection in any outbreak of diphtheria by bacteriological examination of the throat alone must, I think, be apparent from the above observations. The remarks made at the close of the last section, with regard to the significance of bacilli found in the throat, apply, however, equally to the organisms found in the nose.

¹ *Brit. Med. Journ.*, 21st Dec., 1901, p. 1799.

² *loc. cit.*

V. The Prevention of Post-scarlatinal Diphtheria.

This subject divides itself naturally into two parts—the prevention of the introduction of virulent diphtheria bacilli into a ward, and the prevention of spread among patients.

(A.) **Prevention of Introduction.**—Introduction, as has been pointed out, seems to take place through patients who, whether suffering from clinical diphtheria or not, are admitted with virulent bacilli in their throats or noses; occasionally it may occur through members of the staff who have been working in wards containing cases of diphtheria.

(i) *By members of the staff.*—It follows from the observations recorded in preceding sections that the transference of nurses from the diphtheria to the scarlet fever side should not occur more frequently than can be helped, and that those who have been working in wards containing diphtheria or post-scarlatinal diphtheria patients should not be put on duty in scarlet fever wards unless they have been proved by culturing to be free from the means of infecting their charges with diphtheria. It is obvious, as Denny¹ remarks, that a person is dangerous in proportion to the number of bacilli which are given off from him. Thus, in an acute case of diphtheria when the child is coughing and gagging and the secretions are profuse, the bacilli will be disseminated more than they are in mild or convalescent cases. Still from mild cases, and equally from healthy individuals, there is abundant opportunity, as in coughing and sneezing, for the bacilli to be disseminated. A likely means in the circumstances under consideration, both of acquiring and of distributing infection, would seem to be the fondling and kissing of children; the rule, understood in every hospital, that no child should be kissed by a nurse is without doubt very frequently broken.

It is important that in the selection of fever nurses special attention should be paid to the condition of the throat, on account of the relation which appears to exist between it and the period during which infectivity, thus acquired, lasts. Of four nurses, who about the same time, without impairment of health, carried diphtheria bacilli in their throats, one had large, rugged tonsils with some remains of adenoids, while the throats of the others were normal in appearance. The latter were by antiseptic treatment freed from the organism in a few days. The one with the abnormal throat, in spite of over a month's rigorous local treatment, followed by some weeks at the seaside, showed virulent diphtheria bacilli nine weeks after the first examination although

¹ *loc. cit.*

during the whole of this period she had not been in contact with any source of infection.

(ii) *By patients.*—A careful inspection of the throat on admission, with bacteriological examination in cases of doubt, is, of course, customary at all fever hospitals. That a similar examination of the nasal cavities is almost of equal importance is, I think, evident from the series of cases examined at this hospital.

The important question arises as to whether a routine bacteriological examination of all patients should be made on admission. Such an examination of the throat was advocated by Garratt and Washbourn¹ as a method of preventing post-scarlatinal diphtheria. They found, as already mentioned, that of the patients admitted under their care at the London Fever Hospital, from March, 1896, to December, 1898, 1·2 per cent. had in their throats bacilli resembling those of diphtheria. These cases were isolated. In 1896, of 637 admissions, three developed post-scarlatinal diphtheria; in 1897, of 431, one; in 1898, out of 325 patients, none at all; the previous record for the hospital had been, in 1893, of 764 admissions, four; in 1894, of 294, one; and in 1895, when, it is stated, an outbreak necessitated the closing of some wards, fourteen. Nevertheless, Todd² found that this systematic examination of the throats had not prevented the occurrence, in the same wards, of a contagious rhinitis associated with the presence of diphtheria bacilli, which in several cases were proved to be virulent; a circumstance which would suggest the advisability of a similar routine examination of the nose on admission.

In the previous sections, however, the attempt has been made to show that, where the patient brings no history of exposure to diphtheria and presents no clinical evidence of that affection in either throat or nose, the bacilli which may be present are in most cases of a saprophytic and harmless character. It must also be remembered, to look at the question from another standpoint, that a single negative culture is no proof of the absence of diphtheria bacilli. Dr Hill, the Director of the Boston Board of Health Laboratory, states³, with regard to bacteriological examination in suspected cases of diphtheria, that from 5 to 10 per cent. of the cases finally proving positive fail to yield a positive result at the first examination. In the examination of cases admitted into this hospital certified diphtheria, a similar error of about 5 per cent. has been noted in cases where the character of the throat has led to the

¹ *loc. cit.*

² *loc. cit.*

³ *Boston Med. and Surg. Journ.*, 7th March, 1901.

belief that the bacilli finally found have not been acquired in hospital. It appears probable from this that a routine examination by single culture would fail to detect quite an appreciable proportion of the carriers of diphtheria bacilli. Thus the adoption of this method would, on the one hand, lead to the isolation of many patients with harmless bacilli, while, on the other, it would not altogether prevent the admission of patients carrying virulent organisms. That it would lead to greater accuracy in clinical observation is probable, but whether the very considerable labour which a routine examination of the throat and nose entails, when dealing with the large numbers which these hospitals receive, would, on this score, be sufficiently repaid is, at least, open to doubt. In considering the subject it is impossible to avoid being influenced by the fact that post-scarlatinal diphtheria has in recent years ceased to have that dread influence over the course of scarlet fever which it formerly possessed, and that now the cases in which it at all assists in producing the fatal result form but an infinitesimal proportion of the patients treated in these hospitals. As already remarked, at this hospital in 1901 of 3,094 scarlet fever patients under treatment only one died who had suffered from post-scarlatinal diphtheria, and he died of lobar pneumonia a month after the commencement of his attack.

Although belonging more strictly to the next subdivision, another suggestion may here be noticed,—the injection of a prophylactic dose of antitoxin. Park states¹ that at the Foundling Hospital, New York, where in the past diphtheria had so frequently followed measles, it is now the rule, instead of making cultures regularly, to immunize every case with antitoxin so soon as the Koplik spots are noticed. Since the starting of this procedure diphtheria as a complication of measles has been completely eliminated. Professor W. R. Smith², in the Harben Lectures for 1900, speaking of prophylactic doses of antitoxin, suggested their use “in hospitals in the case of those scarlet fever patients exposed to the risk of diphtheritic infection, and who so frequently acquire it, especially in the case of all those sent to the convalescent hospitals.” In the case of scarlet fever patients, however, there are many objections to this method,—among others, the length of the period of isolation, averaging ten weeks, in relation to the often temporary character of the immunity conferred by antitoxin, and the important fact that, by preventing the development of the disease

¹ *Trans. of the Assoc. of Amer. Physicians*, xv., 1900, p. 222.

² *Journal of State Medicine*, viii., p. 182, March, 1900.

it encourages a free distribution of virulent bacilli among the protected patients which on their discharge may work havoc among others.

(B.) **Prevention of spread.**—While bacteriological investigation may, I think, be made subsidiary to the clinical examination in attempting to prevent the introduction of infection, in the prevention of spread, on the other hand, there can be no doubt it should take a very prominent part. When the virulent bacillus has invaded a scarlet fever ward, as evidenced by the occurrence of a case of secondary diphtheria, all the patients should be kept in bed and inter-infection by their toys, handkerchiefs, etc., prevented, while the condition of their throats and nasal cavities is investigated, two rounds of cultures, at least, being made from nose and fauces. Similar cultures should also be made from the nurses. Those in whom diphtheria bacilli are found should be removed to an isolation ward and appropriately treated. When this method is adopted, a ward can usually be safely considered free from infection after only a few days' quarantine. Attention has been directed to the importance of an examination of the nurses and of a supervision over the toys (especially with regard to the slates and mouth-instruments, so frequently supplied by parents), because in several examinations in our wards, in connection with cases of post-scarlatinal diphtheria, these have appeared to be important factors in stamping out infection.

I now come to an important point in the administration of fever hospitals. It is obvious that the number of patients developing post-scarlatinal diphtheria will depend on the number brought in contact with those already infected with the bacillus. When resident medical officer some years ago at a hospital for children, I became convinced that in institutions of that character many-bedded wards were a mistake, on account of the liability to introduction of various infectious diseases. Experience of isolation hospitals had led me to the same opinion as regards fever hospitals. In the designing of institutions to be used largely or entirely for the treatment of children, there can be no question that the smaller the wards, consistent with economy in building and administration, the better.

It naturally follows that the intermingling of patients from different wards is much to be deprecated. At several isolation hospitals in this country a common recreation room is provided for the use of all the scarlet fever convalescents. It is easy to see how this favours the spread of secondary diphtheria: an infecting child is brought into most intimate contact with scores of more or less susceptible patients,

and it is but natural that hospitals of such construction should present a high incidence of post-scarlatinal diphtheria. Again, it cannot be doubted that in hospitals where this general congregation of patients is permitted the production of bacillus-carrying, yet apparently healthy, children is not infrequent. The possible harm which may be done by the introduction of virulent diphtheria bacilli into a ward is to be gauged, not by the number of patients who develop post-scarlatinal diphtheria, but by the number infected with the bacillus. The former, which alone is recorded in a hospital's statistics, is no guide to the amount of evil which may possibly result from the discharge to their homes and schools of children who, though apparently healthy, carry with them the virulent bacillus of diphtheria. It follows that the adoption of what may be called a "barrack" system should be discountenanced in hospitals for fever convalescents, that the children from different wards should be prevented from mixing, and that a diligent search, after the manner suggested, should be made among those known to have been exposed to infection.

Summary of Conclusions.

1. The principal cause of post-scarlatinal diphtheria is the admission into the scarlet fever wards of patients who, whether suffering from clinical diphtheria or not, are carriers of virulent diphtheria bacilli.

2. In large centres of population, where diphtheria always exists, diphtheria bacilli are to be found in a not inconsiderable proportion of school-children. In the absence both of evidence of clinical diphtheria and of a history of exposure to that affection, the bacilli are, in the majority of cases, of the non-virulent or saprophytic type and of little hygienic importance; in cases, on the other hand, where the clinical supports the bacteriological examination the bacilli are almost certainly virulent, and therefore dangerous; while in cases where the patient is known to have been exposed to infection the chances are great that the organisms are of the pathogenic variety, and such cases should always be regarded with grave suspicion.

3. On account of the prevalence of the non-virulent bacillus and the fallacies of single cultures, it may be doubted whether a routine bacteriological examination of throat and nose of all patients on admission would prove of sufficient value to repay the labour involved.

Chief reliance must be placed on a careful inspection on admission, not only of the throat, but also of the nasal cavities, bacteriological examination being resorted to in cases of doubt.

4. In eradicating infection from an invaded ward bacteriological methods are, on the other hand, of prime importance, since children with apparently healthy throats and noses are often found to have acquired the virulent bacillus. An attempt to stamp out any outbreak of diphtheria by bacteriological examination of the throat alone is, however, futile, owing to the frequent infection of the nasal cavities.

5. Fibrinous rhinitis, which appears to be a not infrequent, though often unrecognised, affection of children is a common form of post-scarlatinal diphtheria.

6. Although the treatment of diphtheria in the same hospital has no appreciable influence on the incidence of this complication of scarlet fever, it is advisable that precautions should be taken lest nurses harbouring diphtheria bacilli carry infection from the diphtheria to the scarlet fever wards.

7. To limit the risk of exposure to infection many-bedded wards should be avoided, and the mixing of patients from different wards prevented.

NEUTRAL-RED IN THE ROUTINE EXAMINATION
OF WATER.

BY ERNEST E. IRONS.

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THE use of neutral-red culture media has recently been suggested for the detection of *B. coli* in water supplies. Rothberger⁽¹⁾ grew a number of organisms on media containing various organic dyes, and found that while some species were able to bring about a reduction of the colouring matter in the medium, others produced no change. This difference in reaction was particularly marked in the case of *B. typhosus* and *B. coli* in media containing neutral-red. *B. coli* caused the reduction of the neutral-red to yellow with green fluorescence, whilst *B. typhosus* produced no change of colour beyond an occasional fading of the red. After testing a number of races of *B. coli* and *B. typhosus*, Rothberger proposed neutral-red media for the differentiation of the two organisms. Scheffler⁽²⁾ confirmed in the main the work of Rothberger, and further observed that the reaction is by no means specific for *B. coli*. In addition to a number of bacterial species not commonly found in water, which gave the reaction, Scheffler found 3 of 13 organisms in spring and river waters, and 8 of 18 intestinal organisms from man which, though not belonging to the colon group, gave the neutral-red reaction.

The experiments of Makgill⁽³⁾ showed that neutral-red media form a delicate test for *B. coli* in pure culture in water, but that the presence of other organisms tends to delay the reaction. Savage⁽⁴⁾ examined 44 waters from various sources, using neutral-red media and made at the same time a search for *B. coli*. From the 34 waters with which a positive neutral-red reaction was obtained, organisms identified as *B. coli*

were isolated in 31 cases¹. From none of the 10 negative cases was *B. coli* isolated. Savage concludes that in the series of waters examined, the error of assuming that the yellow colour and fluorescence were due to the presence of *B. coli* was about 5%, and that the test gave approximately accurate results, even if further isolation of this bacillus had not been proceeded with.

If similar results could be obtained with ground and surface waters in general, we should have at our command an additional and valuable method for the detection of *B. coli* in water supplies. In the course of the recent Streams Examination for the Chicago Sanitary District an opportunity occurred for further determining the value of neutral-red in the routine examination of water.

Methods.

Neutral-red agar and bouillon were prepared by adding to neutral agar² or bouillon 0.5% dextrose, and 1% of a 0.5% aqueous solution of neutral-red (Grübler's). In the routine examination, broth was generally employed. In testing the neutral-red reaction of organisms in culture, both broth and agar shake cultures were used. Dextrose broth for the fermentation tubes employed in parallel tests was made by adding 1% dextrose to neutral sugar-free broth. Dilutions of the waters were prepared by means of small 150 c.c. flasks containing known amounts of sterile tap water.

All cultures were kept at 37° C. The dextrose fermentation tubes were examined for gas after 24 and 48 hours, allowed to cool, and the CO₂ absorbed with a 2% solution of NaOH. The appearances in neutral-red tubes were recorded after 24 and 48 hours, 5, 7, and 10 days. Tubes in which the reaction was delayed beyond 3 days gave positive results later in comparatively few cases.

¹ Although it is recognized that organisms occur in water which are intermediate between *B. coli* and allied groups, and that one group passes by a series of intermediate forms into another, nevertheless, ultimate clearness and precision are not likely to be attained by a too liberal extension of the limits of the true *B. coli* group. To this end, a somewhat detailed study must be made of the cultural characteristics of organisms which upon superficial examination appear similar to *B. coli*. Exception may well be taken on this score to the criteria employed by Makgill and Savage for the differentiation of *B. coli* from other water bacteria, particularly as regards the incomplete data given concerning fermentation tests.

² Except where otherwise stated, media were prepared according to the "Procedures Recommended for the Study of Bacteria." *Reports and Papers of the Amer. Pub. Health Assoc.* 1898, vol. xxiii. p. 60.

In the following experiments, made in connection with the daily examination of river waters, neutral-red determinations were made with a number of dilutions of each water, controlled in exact parallel by similar determinations with the dextrose fermentation tube. It has been shown ⁽⁵⁾ that when the dextrose fermentation tube yields approximately 33% of CO₂, *B. coli* is almost invariably present. Table A illustrates the method of comparison employed.

TABLE A.

No. of Sample	Amount of water used	Gas-Production Dextrose Fermentation Tube				Neutral-Red		
		24 hours	48 hours	Proportion of H to CO ₂	Interpretation B. coli present	Reaction in Original Tube	B. coli Isolated	Reaction of Culture Isolated
183	·001 c.c.	no gas	arm clear	—	—	—		
	·01 c.c. (a)	"	arm turbid	—	—	—		
		(b)	"	—	—	+		
	·1 c.c. (a)	"	5% ₀	—	—	+	—	+
		(b)	10% ₀	3 : 2	+	+		+
196	1 c.c.	15% ₀	25% ₀	3 : 1	+	+		
	·001 c.c.	no gas	arm turbid	—	—	—		
	·01 c.c. (a)	"	"	—	—	+	—	+
		(b)	10% ₀	—	—	+		
	·1 c.c. (a)	10% ₀	20% ₀	3 : 1	+	+		
241		(b)	25% ₀	2 : 1	+	+		
	1 c.c.	65% ₀	75% ₀	2 : 1	+	+		
	·001 c.c.	no gas	arm clear	—	—	—		
	·01 c.c. (a)	"	" turbid	—	—	+	—	+
		(b)	" "	—	—	+		+
251	·1 c.c. (a)	"	" "	—	—	+		
		(b)	" "	—	—	+		
	1 c.c.	5% ₀	10% ₀	3 : 1	+	+		
	2 c.c.	95% ₀	95% ₀	3 : 2	+	+		
	·001 c.c.	no gas	arm clear	—	—	—		
	·01 c.c. (a)	"	"	—	—	—		
		(b)	bubble	—	—	—		
	·1 c.c. (a)	"	arm turbid	—	—	+	—	+
		(b)	"	—	—	+		
	1 c.c.	45% ₀	55% ₀	3 : 2	+	+		
	2 c.c.	95% ₀	100% ₀	2 : 1	+	+		

As will appear from the table, such dilutions of the waters were employed that *B. coli* was almost always found in the lowest and rarely in the highest dilution. It will further be noted that from those neutral-red tubes, giving a positive reaction when the fermentation tubes of the corresponding dilution were negative, a careful

search failed to show the presence of *B. coli*, but other organisms were present which in culture gave the neutral-red reaction, thus accounting in each case for the reaction of the original tube. The results obtained with 45 waters examined in the same manner by means of both the fermentation tube and the neutral-red methods are summarized in the table below (Table B).

TABLE B.

Dilutions	.001 c.c.	.01 c.c.	.1 c.c.	1 c.c.	2 c.c.	Totals	% +
Dextrose Fermentation Tube Determinations	+ 1	10	36	39	14	100	35 %
	- 44	80	54	6	1	185	
Neutral-Red Determinations	+ 0	20	54	45	15	134	47 %
	- 45	70	36	0	0	151	

With each method there are shown 285 determinations in exact parallel, with 35 % positive with the fermentation tube, and 47 % positive with the neutral-red method. In so large a number of determinations, this excess of positive results with neutral-red could hardly be due to chance. I have carefully considered the possibility that the excess might be due to the failure of *B. coli* to give a characteristic gas-production in the dextrose tube; but a large number of determinations made in connection with the present and previous⁽⁵⁾ work show that the dextrose fermentation tube gives a fairly accurate test for the presence of *B. coli*. Nor can it be objected that the dextrose reaction is too rigorous a standard for the reason that it excludes organisms giving no gas in dextrose, but otherwise like *B. coli*; for an examination of Table C will show that of 30 organisms (Group V) which do not liquefy gelatin and give no gas with dextrose, only two give the neutral-red reaction. A closer examination of individual tubes and of the neutral-red reaction of a number of cultures of water bacteria will indicate more clearly the source of the excess of positive neutral-red determinations.

Twenty-two neutral-red tubes, which gave positive reactions when the fermentation tubes of the corresponding dilutions were negative, were examined for the presence of *B. coli*. In the case of 2, *B. coli* was found, and from 19 of the remaining 20, organisms not *B. coli* were isolated, which however gave the neutral-red reaction. The organisms giving the reaction in 15 of the above 19 tubes, liquefied gelatin and gave no gas in dextrose broth.

The reactions of 132 cultures of organisms isolated in the course of

the work were observed on the various routine culture media, and on neutral-red agar and bouillon. These cultures all grew at 37° C. and are classified below, taking into consideration their reactions on all the routine media, but with special reference to their growth on those mentioned under the several groups. In the arrangement of these groups, an endeavour has been made to place together those organisms closely allied, although it is of course recognized that Groups V and VI may each contain organisms differing more or less widely in their relationships.

GROUP I. *B. coli*.

(1) Coagulation of milk with formation of acid; (2) non-liquefaction of gelatin and casein; (3) gas in dextrose, lactose, and saccharose bouillon, or in dextrose and lactose, with gas formula showing excess of H (gas not absorbed by NaOH solution).

GROUP II. *B. enteritidis*?

(1) Non-coagulation of milk, with production of alkali; (2) non-liquefaction of gelatin, and casein; (3) gas in dextrose bouillon with excess of H; (4) no gas in lactose or saccharose.

GROUP III. *B. proteus*.

(1) Coagulation of milk, with formation of acid; (2) liquefaction of gelatin and casein; (3) gas in dextrose and saccharose bouillon with marked excess of H; (4) no gas in lactose bouillon.

GROUP IV. *B. cloacae*.

(1) Coagulation of milk, with formation of acid; (2) liquefaction of gelatin; (3) gas in dextrose and saccharose bouillon with excess of CO₂, and in lactose bouillon with varying gas ratio.

GROUP V.

(1) No gas in sugar media; (2) non-liquefaction of gelatin.

GROUP VI.

(1) No gas in sugar media; (2) liquefaction of gelatin.

The reactions of the organisms of these groups on neutral-red media are summarized in Table C.

TABLE C.

Group	I	II	III	IV	V	VI
+ Reaction } Neutral-Red }	26	2	15	14	2	30
- Reaction } Neutral-Red }	1	0	1	0	28	12

It is evident that organisms common in river water other than *B. coli* give the neutral-red reaction under the conditions of the test. Scheffler and Rosenberger⁽⁶⁾ have pointed out that the neutral-red reaction, essentially that of reduction, is, as one would expect from its nature, not specific for *B. coli*. In view of the evident non-specificity of the reaction, the marked excess of positive reactions with neutral-red compared to the corresponding positive determinations with the fermentation tube, is easily accounted for. Although neutral-red gives approximately accurate determinations when only *B. coli* is present, the results obtained in the examination of a water for *B. coli* by the neutral-red method alone, are likely to be misleading, the tendency obviously being to give too high an estimate of the number of the organism present. We must conclude, therefore, that in the routine examination of water, the neutral-red reaction, when used alone, cannot be depended upon for the diagnosis of *B. coli*, since the reaction is given under the conditions of the test by a number of other common water organisms which no classification, however liberal, would place in the colon group.

I am indebted to Professor E. O. Jordan, at whose suggestion this work was undertaken, for many valuable suggestions and criticisms.

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THE SIGNIFICANCE OF BACILLUS COLI IN DRINKING WATER.

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OF late years the bacteriological examination of water has become of greater importance in routine work and more widely employed. With this development it has become increasingly apparent that the mere number of organisms present in a given quantity of water, though of considerable value, is liable to cause many fallacies if solely relied upon.

The *kinds* of organisms present are of greater significance. It is impossible to work out and determine all the organisms present, nor is it necessary, so the organisms especially associated with sewage and harmful contamination in general, become of especial importance. Of such organisms the *Bacillus coli* is considered by most workers to be among the most valuable. In itself as met with in water it is probably quite harmless, but as an indicator of contamination its value has been rated very highly.

As to the exact significance to be ascribed to this organism authorities however differ widely, and the opinions held range on the one hand from those who consider its presence as significant of contamination without regard to the number of such *B. coli* present, to those who reject its detection as of no value because of its wide distribution in nature and the possibility of its being derived from apparently harmless sources.

That such a wide discrepancy of opinion exists is seen when the following results and opinions held by different workers are considered.

E. O. Jordan⁽¹⁾ objects to *B. coli* as a true index of sewage contamination because he has found "in spring water which was beyond any

suspicion of contamination bacteria which in form, size, growth on gelatine, potato, etc. were indistinguishable from *Bacillus coli communis*."

Theobald Smith⁽²⁾ says "it is safe to infer that an organism which is so uniformly present in the intestinal tract, and which possesses to a slight degree pathogenic powers, really belongs there, and that its presence outside of the intestines in soil and water may be regarded as due to the continual contamination with faecal discharges of men and animals. Either through the presence of sewage in the water or through the washing into streams of surface soil from manured ground the colon bacilli enter streams and thus become a valuable index of that kind of pollution which we should most carefully guard against."

Weissenfeld⁽³⁾ found a coli-like organism in good and bad waters, using 1 litre as the standard amount to examine. His cultural and other characters descriptive of *B. coli* are however by no means conclusive.

Other observers lay stress upon the quantitative enumeration of the bacillus. Thus Horrocks⁽⁴⁾ states, "I would say that a water which contained *B. coli* so sparsely that 200 c.c. required to be tested in order to find it, has probably been polluted with sewage, but the contamination was not of recent date."

Pakes⁽⁵⁾ asserts that, "Drinking water from a deep well should contain no *B. coli* in any quantity: water from other sources which contains the *B. coli* in 20 c.c. or less should be condemned; that which contains the organism in any quantity between 20 and 50 should be looked upon as suspicious, between 50 and 100 as slightly suspicious, and only in greater quantity than 100 c.c. as probably safe."

Houston⁽⁶⁾ has quite recently very clearly stated this quantitative view of the matter, pointing out the value of the presence of *B. coli* if the relative numbers present are considered.

Other authors, viz. Chick and Boyce^{(7) (8)}, have contented themselves with examining small quantities only of water, and base their views on the presence or absence of this organism in 1 c.c. of the water. With such divergent views there is obviously room for careful, renewed investigation.

In such an investigation the three following points must, in my opinion, always be kept in view:—

(1) That it is not so much the presence of *B. coli* which is of possible value but the *number* present.

The consideration of the problem is essentially quantitative, and the

number of *B. coli* in a standard amount of water must be considered. This view is insisted upon by many English bacteriologists and there can be no doubt of its vital importance in studying the question.

(2) That the kind of water in which this organism is found is of great importance, and that a number of *B. coli* per litre may be passed as safe, or at least as no evidence of dangerous contamination, in a water from one source which would be sufficient to condemn the water from another source.

(3) The exact point on the water supply from which the sample is collected is of great importance. Frequently results differing considerably are obtained with samples taken from source and tap respectively, even with quite unfiltered waters; and with arbitrary standards one would be condemned and the other passed as satisfactory. Illustrations of this are furnished in the following tables.

This factor though very generally known is frequently ignored by many of those writing on this question.

To study the subject to the best advantage it is necessary to have full and reliable information as to the source and nature of the water of which samples are examined.

Chemical analyses of an exactly similar sample collected from the same place and at the same time are also of great value for comparison. In other words, all three methods of examination should be employed, i.e. the sanitary, the bacteriological, and the chemical.

For all the waters referred to in this paper very great care was taken to obtain satisfactory information as to the exact nature of the water supply, its possibilities of contamination, and any particulars likely to be of use in the investigation. A considerable number of samples are not included because full particulars were not available.

I personally inspected and investigated several of the most important water supplies, taking my own samples.

The information for the others was derived from reliable sources, and I wish here to record my thanks to Dr W. Williams, Medical Officer of Health of the Administrative County of Glamorgan, for the trouble he has taken in supplying me with much information in regard to the water supplies examined, and for the care which he has taken to verify any doubtful points, such as exact source, or possibilities of contamination. His unrivalled knowledge of the water supplies of Glamorgan has been of great value in obtaining accurate information. The chemical analyses were all performed in the Cardiff and County Public Health Laboratory, some of them by myself but the majority by Mr. Sugden,

B.Sc., F.I.C. Assistant Bacteriologist, to whom I am indebted for much careful and accurate work.

To arrive at the significance of *B. coli* in water I have studied a large number of public supplies, at different times and taken from different parts of the water system. Local wells and other small supplies have also been investigated.

The method used to examine the samples for *B. coli* only requires a brief notice here as it is fully described elsewhere⁽⁹⁾. It consists in incubating varying quantities of the water at 37° C. with ordinary broth containing $\frac{1}{2}$ per cent. glucose and 1.2 per cent. of a $\frac{1}{2}$ per cent. watery solution of neutral-red.

If *B. coli* is present the red changes to yellow or orange and a marked fluorescence develops. To isolate the organism and to make certain that it was present, in all but a small proportion of cases the yellow fluid was brushed over agar or gelatine plates and the *B. coli* isolated and its characters worked out.

In all cases except those otherwise indicated this is the course that has been taken. If no reaction developed in any of the tubes the largest amount of water was in the same way brushed and examined for *B. coli*, in all cases with a negative result.

As a rule, but not quite invariably, the largest amount of water (*i.e.* the 40 c.c.) was brushed when a complete negative result was obtained, and the smallest amount giving positive result (*i.e.* change to yellow and fluorescence) for the waters giving a positive reaction.

It should also be stated that for the bacteriological examinations very great care was taken to have media of standard reaction.

Both the agar and gelatine were standardised to a +1.0 per cent. reaction (as recommended by Dr Eyre)⁽¹⁰⁾.

The agar plates were counted after about 40—46 hours' incubation and the gelatine daily for as long as possible (*i.e.* until liquefied). This usually represented a 3 to 4 days' count.

The different supplies examined, with data as to their nature and possibilities of contamination, and results of chemical and bacteriological examinations, will be considered first. Pure upland surface waters will be first described.

Supply No. 1. This is the most important public supply in Glamorganshire. The water is a typical upland surface water, the gathering grounds being in Brecknockshire, quite near the Brecknock Beacons and over 30 miles from the town supplied.

The collecting areas are two in number. The larger (Reservoir A) has a water

collecting area of 51 acres ; and the reservoir, which is at an elevation of 1340 ft., has a capacity of 345 million gallons. The area of the other collecting area is 45 acres ; and the reservoir (Reservoir B), which has an elevation of 1073 ft., has a capacity of 322 million gallons.

The water is carried from these (excluding the several balancing reservoirs *en route*) to two large storage reservoirs a few miles from the main town supplied. The distance from the lower collecting reservoir (Reservoir B) to the storage reservoir is 32 miles. The storage reservoirs have a capacity of 317 million gallons (Reservoir C) and 80 million gallons (Reservoir D) respectively. From these the water passes to the filter-beds (at E and F), where it is filtered through sand and then distributed.

The E. filter-beds are 6 in number, each capable of filtering 1 million gallons per day. At F they are 5 in number. The water is screened a large number of times before the final filtration. The filtration cannot be considered very efficient. When the sand becomes dirty and the filtration slower the top layers are removed and washed. In other words the filtration is mainly mechanical and only to a small extent *vital*.

With regard to possibilities of contamination, the gathering areas are among the Brecknock hills and quite remote from all habitations. The nearest town is 6 miles away, and within that distance there are only a few scattered farms. There is only one house on each gathering area. For the lower one this is the house of the waterworks keeper ; earth-closets are used and the most rigid precautions are taken. Contamination from this source is impossible. On the upper reservoir (Reservoir A) there is only one house, and the drainage of this certainly finds its way into the reservoir. This is the only possible source of contamination to either reservoir from human sources. It is a very small one, and the results of the examinations made show that it is quite unimportant as regards the points at issue.

The gathering areas are ordinary hill side, and there are *no* manured fields or cultivated land on them. The entering streams rise in the hills round and can be traced to their sources. The gathering areas are undoubtedly among the best in the country, with no possibility of human contamination except the small source indicated.

Sheep however are on the gathering area all the year round. The two lower storage reservoirs (C and D) only contain the water from these two collecting reservoirs ; *i.e.* all the streams around them are very carefully led away and prevented from entering these reservoirs.

Wild duck and other birds can frequently be seen in large numbers on the water. There is a probability of contamination therefore from animal sources.

I have personally inspected all the reservoirs, gathering areas and filter-beds and satisfied myself as to the correctness of the above description.

The significance of *B. coli* in such a water is therefore a fairly straightforward one, and a large number of samples were examined. The results obtained are given in Tables I, II, and III. In Table IV a selection of the results of chemical analyses of this water is given for comparison.

Supply No. 2. Also a typical upland surface water and an important public supply, supplying a population of about 100,000 people. The gathering area is in Glamorganshire, at a considerable elevation. The main reservoir is formed by a dam on a small river, which rises some distance further away among the hills,

quite away from possibilities of human contamination. The gathering area has only one house on it, which is $\frac{1}{2}$ a mile *below* the upper reservoir, is used by the two men connected with the waterworks, and from which contamination is rigidly excluded. The surrounding hills have only a few tracks and no roads, and are only used for sheep. There are no manured fields or cultivated land. By an overflow weir a large quantity of water passes out from the main reservoir, and makes the river. This river is again partially blocked about $1-1\frac{1}{2}$ miles lower down to form another reservoir. The water from this lower reservoir is filtered through sand in beds quite near by and is then distributed. The water from the upper reservoir passes by pipes to near the sand filter-beds, where it is filtered through quartz-filters, the water passing into a covered reservoir.

This gathering area was personally inspected by Dr Williams and myself in Dec. 1901. The only possible source of contamination was from the excreta of animals or it may be from the soil. Sheep were allowed and could be plentifully seen all over the gathering area; and considerable quantities of sheep dung were seen, some quite close to the water and washed by it. A number of samples of water were collected (Nos. 8—14, incl. Table V), No. 8 from the upper reservoir, No. 11 from the river midway between the two reservoirs, Nos. 10, 12, 13 from different little mountain streams the sources of which could be readily traced some distance away among the uplands. All three entered the river between the reservoirs. No. 14 was collected *in* the quartz-filters just before filtration, and No. 9 from a little patch of marsh water (there had been much rain a few days previously) about 200 yards below the upper reservoir and a few yards from the river.

A sample of soil was also collected from just by the side of No. 12 stream. The soil was moist and peaty and contained much vegetable matter.

The results obtained from these and other samples are given in Tables V and VI.

Supply No. 3. This is a proposed water supply. The samples were collected by Dr W. Williams, to whom I am indebted for the following description.

The proposed collecting area is 2300 acres, and consists of a natural basin through which the river runs. The area is entirely on old red sandstone and there is no peat. The rock appears on the surface everywhere and along the bed of the river. The basin is bounded by hills, under the crest of which appear numerous springs. The sides of the basin are markedly furrowed by springs, the water of which flows into the river. The river rises at the head of the basin in a number of small streams at an elevation of 1500 ft. The area shows evidence of cow dung and plenty of sheep dung. Sheep and other animals are allowed on all the summer. Samples collected Jan. 13th, 1902.

There are no houses on the area, and Dr Williams informs me that apart from animal excreta there is no possibility of any contamination and that it is one of the very best gathering areas with which he is acquainted. Six samples were collected: No. 1 and No. 2 from the river on each side of the gauge. No. 4 from the river at the top of the proposed reservoir, *i.e.* about $\frac{3}{4}$ mile above the gauge. No. 3 was from a streamlet having its origin in a spring about 400 yards away. As collected, the sample consisted mainly of spring water but with some upland surface water. No. 5 and No. 6 were pure spring waters collected from the upper part of the gathering area from 2 springs. These samples were collected as the water came out from the ground. The results obtained are given in Table VII.

No chemical examinations were made.

A number of other entirely upland surface waters were also examined but not in such detail, so that a shorter description will answer every purpose. These waters include Nos. 4 to 9 inclusive; and the results of the bacteriological and chemical analyses are given in Tables VI, VIII, IX and X.

Supply No. 4. Not a large supply. Supplies a population of about 8000. A very limited gathering area from which the upland surface water collects into a reservoir. This is a few miles from the town. One small house only on the gathering area, but no cultivated land. Soil very peaty, and sheep and other animals graze on the gathering area. The water is not filtered.

Supply No. 5. An upland surface water collected from peaty mountain land. There is one farm-house and a few manured fields on the area, and numerous sheep graze over it. The area is surrounded by trees and by vegetation, and cannot be considered a good gathering area. There are also a number of old colliery workings which may contain excrement. Water is sand-filtered.

Supply No. 6. An upland surface water entirely, all the water being derived from the surface of hills. The whole of the gathering ground is entirely devoid of dwelling-houses. A few mountain paths traverse the collecting area, and are not infrequently used by colliers and others. The uplands serve as grazing ground for hundreds of sheep, which are the only quadrupeds to be found on the whole area. Before distribution the water is filtered through sand. The subsoil of the collecting area is carboniferous limestone, and distributed over the area are a few very small "pockets" of peat.

Supply No. 7. An upland surface water with collecting area of about 480 acres. Water liable to pollution from manured and ploughed fields or a few farms on the area. Water has often a distinct yellow tint, and the storage reservoir has a thick layer of peat at the bottom. Filtered through sand before distribution.

Supply No. 8. An upland surface water which is collected into an open reservoir and then distributed. Sand-filtered before distribution. A certain amount of spring water also is collected into the reservoir.

Supply No. 9. A large upland surface water which supplies a population of over 100,000 people. It is of interest in being an upland surface water undoubtedly exposed to contamination. There are three collecting reservoirs, of which the two upper, A or B, are several miles above the lower, and are not liable to any pollution other than from sheep. The third reservoir (Reservoir C) is on the same river but lower down, and is liable to distinct contamination from four separate farms. From all the four farms the contamination is distinct and gross in nature. Thus for one, a stream feeding the main river passes through the farm-yard, where there are accumulations of manure. For two others there are accumulations of manure within a few yards of streams feeding the main river; while for the fourth, not only does manure drain into a feeding stream but also the contents of the slop water drains. The river receives all but one of these streams before it is again blocked to form Reservoir C; which reservoir also receives direct the fourth contaminated stream.

The water from the two upper reservoirs (A and B) is filtered about six miles from the source and supplied to one part of the town. The water from Reservoir C is also filtered about six miles away and supplies the rest of the town. I am

indebted to the Medical Officer of Health of this town for full particulars and plans of the different streams and reservoirs.

A number of water supplies were also examined which are neither pure upland surface water nor entirely spring water, but which are mainly springs, but supplemented by upland waters. Particulars of a few of these are given briefly. The results of the bacteriological and chemical analyses are given in Tables XI and XII.

Supply No. 10. Partly a spring, partly an upland surface water from the hill sides. Collected direct into a reservoir but not in any way filtered. The samples were taken from a tap about two miles from the source. Sheep over the hills. A small supply.

Supply No. 11. Another small supply, partly upland surface and partly from some small springs. The water is filtered through about 3 ft. of gravel and sand, and is then stored in a closed tank before distribution. The gathering area is partly peaty and is used for sheep, but is quite free from manured or cultivated fields.

Supply No. 12. This water supply consists of spring and upland surface water. The water from the several springs runs in a channel or brook for about a mile before it enters the service tank from which it is distributed. In its passage along the channel the water is supplemented by streams from the surrounding hills. On these hills there are a few sheep and sometimes a few horses and cattle but no cultivated fields. No possibility of sewage or human contamination. The water is not filtered in any way.

Supply No. 13. A quite small supply. Upland surface and spring. Sheep the only possible contamination. Unfiltered.

Supply No. 14. Another small supply. Mainly springs on mountain side. A small mountain stream is also taken in. Filtered through sand-filter. Sheep on the uplands as usual, and this the only possible source of contamination. No houses or cultivated land.

Pure Spring or Deep Well Waters.

Not a large number were examined and worked out, if a large supply of doubtful origin (No. 20) is excluded. The results obtained are given in Tables XIII and XIV.

Supply No. 15. This water supply is obtained from two springs in pennant sandstone, the water being impounded into a properly constructed reservoir. The reservoir is a covered one and no surface water can obtain admission into it. A pure spring water. It is distributed unfiltered.

Supply No. 16. This small supply is obtained from a spring, and no surface water gains access to it. The water is conveyed from the source by cast-iron pipes to a storage tank. It is not filtered in any way and there are no possibilities of contamination from human or animal sources.

Supply No. 17. Under this head are included a number of isolated supplies. Each was only examined once, *i.e.* each represents a quite separate supply. A considerably larger number of springs were examined, but only those in which full particulars were obtained and in which the examination for *B. coli* was thoroughly worked out are included.

Shallow Wells.

Only those are mentioned for which particulars are available. Under supply No. 18 are grouped a number of separate supplies. The numbers correspond to the numbers in Table XV.

Supply No. 18. (1) A shallow well said to be about 30 ft. deep. It is situated close to a chapel burial-ground and therefore regarded with suspicion. The strata consist of a loose sandstone gravel with a few beds of clay. I am informed that the water has been analysed two or three times during the last three years and the report has always been favourable.

(2) and (14) A surface well about 30 ft. deep. Surface water said not to gain access. Has a pump. Possibilities of contamination present.

(3) A shallow well in a peaty soil and close to the road. Known to be liable to contamination.

(4) A well in a clay soil. Probably polluted from slop water.

(5) A surface well, said to be liable to organic and vegetable pollution.

(6) A well, probably a shallow one. Walls not properly built and surface water not kept out. Nearest house 60 yards.

(7) A shallow well. Collected from pump.

(8) A shallow well situated upon a common. No houses near, and no liability to contamination. Properly covered in and a pump fixed.

(9) An open and shallow well.

(10) An old well about 15 ft. deep in a gravel soil. A row of eight houses near the well and a slop drain passes within three yards of it. Old privy pits 50 yards away. Covered over but surface water gets in.

(11) A well water. Doubtful if a shallow or deep well, but surface washings can and do gain access.

(12) A surface well in a clay and peat soil. Water examined because three Enteric Fever cases among those drinking the water.

(13) A well used by two persons suffering from Enteric Fever. The well is in the middle of a farm-yard. The sides and roof are defective and pollution can take place.

Supply No. 19. An important shallow well supply. The water is conveyed direct from the well in 2-inch pipes to a pump by the Town Hall, a distance of about 250 yards. Surface water is received only when the spring is disturbed by floods. No known sources, I am informed, of contamination, human or animal.

Supply No. 20. This supply will be considered by itself, as it is of a somewhat peculiar nature. It is a large supply and serves about 30,000 people. An underground supply tapped by a well 32 ft. deep and by a long (200 yards) lateral heading from this. The heading runs 25—30 ft. under the surface. The average quantity supplied per day is about 600,000 gallons. The water is pumped from the well into three reservoirs and is then distributed. It is not filtered in any way.

This supply cannot be said to have a gathering area, but the ground over the heading and round the well and pumping station is liable to flooding from a brook which runs in a semicircular fashion round it, and distant about 1200 ft. from the pumping station. This brook is contaminated by houses higher up and its chemical and bacteriological analysis shows marked evidence of pollution.

This area is usually moist, shows marked vegetable growth (grass, etc.), and is in winter frequently flooded and under water for days together. Also cattle and other animals graze over this area during part of the year (it is now enclosed and no animals allowed since October 1901). The exact classification of this water is not easy to decide. It is said to be a deep well water, though it is more correct apparently to call it an underground river. The well is sunk for 32 ft. and the soil from above down is surface soil, blue clay, peat, blue clay, red marl with stone and clay, hard limestone with banks of yellow marl.

The significance of *B. coli* in upland surface waters will first be considered. For this purpose supplies Nos. 1 to 9 are available. It will be noticed that this organism is present in all of them and in the great majority of the samples examined. It is further often present in considerable numbers. The source of these *B. coli* is more readily studied by considering the examinations made at the sources of the different supplies.

In supply No. 1 it will be seen that out of 17 examinations at the source (Table I) this organism was found and isolated in all but 3 (or 4 if a doubtful *B. coli* is excluded) when 40 c.c. was the amount examined: in 9 samples it was found in 10 c.c.; and in 2 c.c. in nearly half the samples in which that amount of water was examined. It was also found in 1 c.c. but never in $\frac{1}{2}$ c.c. It was present in both reservoirs and in all the entering streams examined.

In supply No. 2, six samples were in connection with the source (i.e. Nos. 8, 10, 11, 12, 13, 14), and *B. coli* present and isolated from five. The small entering upland streams are of particular interest as they could be traced to their respective sources. In one of them *B. coli* was present in 2, 10 and 40 c.c., in another in 40 c.c. only, while in the third it was absent in the 50 c.c. examined.

Supply No. 3 gives the results of examinations made of a proposed supply, one of exceptional purity or freedom from contamination. Even in this water *B. coli* was isolated in 4 out of the 6 samples, and in one was present in as little as 2 c.c. It is noteworthy that the two waters in which it was absent were both pure springs free from admixture with upland surface water.

The results of the examination of the sources of other upland surface supplies gave very similar results; and the investigation of samples other than at the source shows also how comparatively numerous this organism may be in this class of waters.

Supply No. 9, a large and important supply, gives a still larger number of *B. coli* present in the different samples. This supply however shows on inspection distinct possibilities of contamination

from farms in the neighbourhood of the reservoirs, and in the gathering area.

B. coli, then, appears to be habitually present in upland surface waters if a sufficient quantity of the water is examined, and further it is not infrequently present in considerable numbers and that in waters which appear to be absolutely free from sewage or human pollution and which on chemical analysis show no evidence of contamination.

What is the source of these *B. coli*? Bacilli so constantly present cannot be fortuitous. They are not natural constituents of water. Their presence can only be due to contamination of the water from some source in which they are numerous.

The possible sources of such contamination can only be the following:—

- (1) From human or sewage pollution.
- (2) From the washings of cultivated soil and manured fields.
- (3) From the washings of ordinary uncultivated upland soil.
- (4) From the excreta of animals grazing on the gathering grounds.

Regarding the first three supplies as being more completely investigated than the other sources, (1) and (2) can be quite excluded considering the nature of the gathering areas, while many of the small streams were traced to their source and shown to be quite free from the possibilities of such contamination. With regard to the possibility of *B. coli* being derived from uncultivated upland soil Houston (11) in his extensive investigations on this bacillus in soil says his results "seem to show conclusively that *B. coli* (or its close allies) is not discoverable or is present in small numbers only in *pure* soils. Further they would seem to indicate that *B. coli* is not readily isolated even from soils polluted with objectionable animal matters unless indeed the contamination is gross in amount and of *recent* sort." In another report the same author states that soil recently polluted with faecal matter will yield *B. coli* in washings therefrom, but other sorts will not yield *B. coli* in any large numbers.

In 15 samples of moorland soil H. Chick (7) found *B. coli* absent in all in the amounts examined, *i.e.* 0.1 to 0.02 gm. In the soil from supply No. 2 (personally collected, *vide supra*) a typical *B. coli* was isolated from 1 loop of the soil. The sample was taken after the upper layer had been rejected, but was quite close to a mountain stream (sample No. 12) and was very moist.

Examination of a small number of pure and contaminated soils for this organism has given me results somewhat similar to those of

Dr Houston, and it may probably be taken as fairly assured that if present in ordinary hillside soils this organism has been derived from animal excreta.

The source of the *B. coli* in these waters can with considerable certainty be ascribed to contamination of the water with animal excreta either directly or indirectly through the intermediary of the soil. Sheep are allowed to graze on all the gathering areas with which I am acquainted, and in all waters from such areas *B. coli* are present, often in considerable numbers. The excreta of sheep can usually be seen all about such water supplies and frequently washed by the water. Such excreta teem¹ with *B. coli* indistinguishable as far as I am aware from *B. coli* obtained from other sources. Five *B. coli* isolated at different times from such sheep dung showed characters quite like those of the ordinary *B. coli* found in water.

Sample No. 9, supply No. 2 (Table V), is of especial interest. The sample was personally collected from a patch of marshy water a few yards from the river. *B. coli* was present in 2, 10 and 40 c.c. Unfortunately smaller quantities were not examined. The considerable number of *B. coli* in this marsh water gives some clue as to the probable method of multiplication of this organism and how it gains access to the water.

Sheep dung is deposited in the stagnant water and such a water rich in organic matter soon swarms with *B. coli*. The next rain washes these into the nearest streamlet and so into the reservoir. The experimental possibility of this occurrence was demonstrated in the laboratory. A 5 litre glass jar was plugged with cotton-wool and sterilized, 4 litres of tapwater were added and the whole again sterilized. About 1½ grm. of sheep dung was added and the jar kept in the outside air and the temperature taken daily. Weather very cold with nightly frosts and the maximum temperature of the experiment about 9° C. Examined after a week and after a fortnight, and very numerous *B. coli* found in the water.

After these organisms gain access to a water supply they probably do not multiply but tend to gradually die out, presumably from the influence of unsuitable environment, the action of gravity, and the competition of the ordinary water micro-organisms.

That this is probable is shown to a certain extent by the results of the analyses. Thus for supply No. 1 the number of *B. coli* as well as

¹ Only one enumeration of the number of *B. coli* in sheep dung was made. In this case it was found that 1 grm. of the fresh dung contained about 280,000 *B. coli*.

of other organisms is generally less in the lower storage reservoirs than in the collecting reservoirs. Also if this organism was capable of considerable multiplication in stored water it is to be expected that the number of *B. coli* in the reservoirs would be much greater than in the main entering streams; but this is not so, the results of the analyses made of such streams showing sometimes more *B. coli* and sometimes fewer. Further, the result of an experiment made with Reservoir C negatives the probability of multiplication. On March 1st, 1902, I collected three samples from Reservoir C, taken respectively, No. 1 from the inlet, No. 2 from the middle (1 foot below the surface), and No. 3 from the outlet. The result of the examinations gave the following figures.

	No. 1	No. 2	No. 3
Developing at 37° C.	28	2	7
" " 20° C.	590	128	121
Number of <i>B. coli</i>	In 2, 10 & 40 c.c.	In 40 c.c. not in 2 or 10 c.c.	Not in 2, 10 or 40 c.c.

The few laboratory experiments made (six in all) confirm this as far as artificial conditions are available for comparison. Further experiments are being carried out. They show that *B. coli* (cultures recently isolated from water and from sheep dung were used) kept in sterile water in Winchester quart bottles plugged with cotton-wool at the outside temperature undergo little or no diminution in number for 48 hours, but at the end of a week there is a great diminution in number, which is still more marked at the end of 2 weeks. This was observed whether the water was a pure filtered water or contained a considerable amount of vegetable organic matter (*e.g.* a peaty water). The diminution in numbers appeared to be still more marked when the ordinary water organisms were also present.

The presence of *B. coli* in spring waters will next be considered (see Tables XIII and XIV). Not a large number of samples were examined, but they were derived from 8 distinct supplies. In only a single instance was *B. coli* isolated from the water in 50 c.c. In one other sample a positive neutral-red reaction was obtained, but no *B. coli* could be isolated though probably present. It is also to be noticed that in Table VII the two samples in which no *B. coli* were present were pure spring waters. Supply No. 20 possesses peculiar features and will be considered by itself.

It is of interest to compare with these results those from water supplies which are mainly spring water but into which a certain amount of upland surface water gains access. Examples of such waters are given in Tables XI and XII.

The results obtained are very similar to those of spring water alone, but as might be anticipated *B. coli* are slightly more frequently found.

The greater the amount of upland surface water the greater the probability of finding *B. coli* in the amounts examined.

B. coli in shallow wells.—This organism is present in almost all, and usually in considerable numbers. The majority were liable to pollution. No. 19 however is said not to be liable to pollution.

What is the significance to be ascribed to the presence of *B. coli* in these different classes of waters?

In entirely spring water my results agree with those of other workers and it seems a fair standard to expect that *B. coli* should not be discoverable in at least 50 c.c. of the water.

In shallow wells their presence must indicate either surface water and washings gaining access, or insufficient filtration through the soil. When the source of such water and its possibilities of contamination are considered it seems not unreasonable to regard their presence in anything like large numbers with much suspicion. The interpretation must be undertaken with a knowledge of the possibilities of contamination and other points of importance. I am averse to arbitrary standards, but the discovery of *B. coli* in as little as 10 c.c. would raise suspicion; and finding them in 2 c.c. would certainly lead me to pronounce strongly against the suitability of the water for drinking purposes.

The significance of *B. coli* in upland surface waters is a matter of considerable difficulty, but one of great interest. In my experience they are present in *all* upland surface supplies and usually in considerable numbers. This is quite apart from possibilities of contamination from human sources, and must be ascribed, as already explained, to contamination from animal excreta.

Can such pollution be considered harmful?

Sheep are allowed to graze on many of the best gathering areas in the country. Sheep and other animals do not suffer from enteric fever or other specific disease transferable to man by water, and it is difficult to see how their presence can be looked upon as harmful.

Professor Boyce remarks (8), "Although the *B. coli* is normally found in the intestine of man and animals, and therefore cannot be

said to be under these circumstances harmful, nevertheless cases do occur in which marked diarrhoea is found associated with great development of this organism in the intestine."

There is however no evidence as far as I am aware that *B. coli* as such and present in water has set up disease by its ingestion, while in Glamorganshire many millions of *B. coli* are daily consumed with no apparent harm.

Houston (6) states, "It cannot be denied that *B. coli* is present in the evacuations of many animals, but we have yet to learn that the excreta of animals are altogether harmless to man." Also Horrocks (12) remarks, "It is not justifiable to assume that the excreta of animals are harmless to man; and in any case a water so polluted could not be considered desirable for drinking purposes."

These remarks appear to me to beg the question at issue.

The value of the detection of *B. coli* in water is pre-eminently that it is *an indication* of contamination by sewage or other material which may contain the actual micro-organism of specific diseases—more particularly the *Bacillus typhosus*.

It is generally accepted that it is not the *B. coli* which are themselves harmful but that they merely serve as indicators of possible contamination with specific organisms.

In upland surface waters my figures point to the conclusion (as far as they go) that *B. coli* cannot be considered such an indicator for these waters, and so much of its value falls to the ground.

Its presence in upland surface waters even in large numbers (*i.e.* 500 per litre) may, and apparently not infrequently does, indicate contamination by animal excreta, a contamination possibly objectionable but causing and indicating danger in no way comparable to the danger caused by contamination with sewage.

I am of opinion that it is particularly unreliable to adopt any arbitrary standard for *B. coli* in upland surface waters. Each case must be considered on its merits.

To state, for example, that because of its proved presence in say 10 c.c. of a water that water should be condemned as showing dangerous contamination would in my opinion be a very unreliable and an unjustifiable deduction, and would, at least for Glamorganshire, condemn many of the best waters in the country.

It is to be noticed also that with the same water (*e.g.* the same reservoir) this organism has been present sometimes in 2, 10 and 40, while at others absent in these amounts or only present in 40 c.c.

while the supply itself has remained free from possibility of contamination other than from animals grazing.

Also the number of *B. coli* varies in different parts of the same reservoir (*vide supra*, for Reservoir C, inlet, middle, and outlet samples). Here arbitrary standards might condemn at one time and not at another, at one part of the reservoir and not at another.

The examination for this organism in upland waters has, I believe, its value, but less than is usually ascribed to it. When found in large numbers such as several per c.c. it may be justifiable to condemn the water as unsuitable for drinking purposes, but if in smaller numbers such a deduction is one not to be made lightly, and may easily be unjustifiable. Thus for supply No. 9 the tables show that *B. coli* was more numerous than in pure upland waters such as Nos. 1—3; but the numerical difference was not sufficiently marked to enable, from this factor alone, a deduction to be made that one water was good and the other bad.

There are some further points of importance which may be mentioned. Supply No. 20 shows features of interest. In supply No. 20 *B. coli* have almost invariably been found, often in very considerable numbers. This supply is said to be a deep well or spring, and there is no doubt that in the sense of the well passing through impermeable strata this is the case.

The source of the very large quantity of water available is somewhat of a mystery. The statement has been made, but I cannot be certain as to its accuracy, that since so much water has been pumped from this supply many of the surface wells in the neighbourhood have become dry. Two examinations made of the soil are of interest. Both samples were collected with proper precautions 6 inches beneath the surface. One sample was taken from near the end of the heading near the Penstock chamber, and therefore from soil liable to flooding from the contaminated brook. The other from soil of the same nature but not liable to flooding. The first sample (from near Penstock) showed about 3,280,000 organisms and *B. coli* was readily isolated (about 400 per gramme), while the control soil showed about 1,360,000 organisms and no *B. coli* were found (in 0.025 gm. examined¹).

¹ More recently (April 14, 1902) 2 fresh soil samples were examined for *B. coli*. In sample *A*, taken from over the heading 6 inches beneath the surface, *B. coli* was isolated from 0.005 gm. of soil, and in sample *B* some distance away it was found in about 0.25 gm. of soil but not in 0.005 gm.

Turning to the bacteriological examinations an obvious feature is the large number of organisms present, and particularly the extraordinary fluctuation in the number of organisms.

This is probably to be ascribed to the suction action of powerful pumps. A further point is that the Bismark-brown *Cladothrix* (Houston) is present not infrequently in this water, an organism rare in most waters.

There seems reasonable ground then for believing that this water, whatever its exact nature, is contaminated by surface water unpurified by filtration through sufficient soil, and by surface water from undesirable sources. I have repeatedly condemned this water on these grounds. The comparison of the chemical and bacteriological analysis for this water is particularly instructive. Chemically it is a water of extreme hardness, but organically it shows no evidence of contamination. The free ammonia is very small, while the albuminoid ammonia, though larger than usually met with in deep well waters, is not high. The chemical results show a very considerable uniformity, though the analyses recorded extend over $1\frac{1}{2}$ years. This is in marked contrast to the bacteriological results. It is however to be noted that if the 6 samples are compared in which chemical and bacteriological examinations of identical samples were made, there is an almost exact parallel between the number of organisms present and the organic purity as shown by the two ammonia figures.

In regard to chemical *versus* bacteriological examination it is not my purpose here to make any elaborate comparison. A large number of analyses are available the majority of which are taken under strictly comparable conditions, *i.e.* at the same time and from exactly the same place. In general it will be noticed that the chemical figures show much less fluctuation and variation from season to season, and the method appears to be much less sensitive.

A water which is contaminated sufficiently to yield evidence of such pollution by chemical analysis will usually show overwhelming evidence pointing to the same conclusion on bacteriological examination, while many waters on the other hand show pollution by bacteriological methods which on chemical analysis alone are above suspicion.

The bacteriological data require however much greater skill and experience to interpret, while the possibility of false deductions from faulty collection or local contamination is very much greater.

A criticism not infrequently applied to tabular results as given

above is that the organisms which are called *B. coli* are not all really that organism but include many organisms present often in good waters and not significant at all of contamination. Thus Horrocks (*loc. cit.* p. 104) says, "The statement that *B. coli* exists abundantly in all waters and soils appears to be based on a very elastic interpretation of the characteristics of *B. coli*."

To answer such a criticism the organisms isolated were all sufficiently worked out, and it is to be noticed that *B. coli* was isolated in all cases unless otherwise indicated, and that all negative neutral-red results were examined in exactly the same way as positive ones to be certain of the absence of the organism in question.

The characters of the *B. coli* isolated are indicated by the small letters in the column of the tables marked "Characters of *B. coli* isolated." They all had certain characters in common.

Under group *a* are included quite typical *B. coli*, *i.e.* organisms which give typical or at least possible surface colonies on agar or gelatine plates, which do not liquefy gelatine, but grow readily on a gelatine slope, generally as a bluish semitranslucent growth, which have a possible morphology (bacilli with rounded ends, and mostly quite short bacilli), are motile, usually very sluggish but occasionally more actively motile, which give uniform turbidity in peptone broth, and which give *all* the three following chemical tests, gas production in glucose media (agar-shake preparations used), milk coagulation with acid production, and the development of indol.

Under group *b* are included organisms quite similar to the above but which produce no gas in sugar media; under group *c* as above, but do not coagulate milk; and under group *d* also as group *a* except that no indol is produced.

Under group *e* are included the organisms which do not fall into any of the above groups and which are doubtful *B. coli*.

An examination of the Tables given shows that out of 95 *B. coli* isolated, 71 are included under group *a* (74·7 p.c.), 1 under group *b* (1·05 p.c.), 14 under group *c* (14·7 p.c.), 7 under group *d* (7·4 p.c.), and 2 under group *e* (2·1 p.c.).

Of these I think all bacteriologists would accept groups *a*, *b*, *c*, and *d* as *B. coli*. The group *e* are however doubtful, so their characters are given briefly.

Supply No. 1, No. 5. Short bacilli with rounded ends; not stained by Gram's method. No spores. Sluggishly motile. Uniform turbidity in peptone broth, on gelatine slope a white growth showing no lique-

faction. Grown in litmus milk, acid production but no coagulation (3 weeks), glucose neutral-red shake, no gas and no neutral-red reaction, Lactose agar-shake gives a small amount of gas, neutral-red broth no colour changes. Tested for indol in 10 days' old peptone broth it gave a slight red reaction.

Supply No. 6, No. 4. Short bacilli, not stained by Gram's method. No spores. Fair motility. Uniform turbidity in peptone broth. On gelatine slope gives a white translucent growth with no liquefaction. Grown in litmus milk, acid production but no coagulation (4 weeks). Glucose neutral-red shake, no gas and no neutral-red reaction but produces gas in lactose agar-shake preparations. No indol reaction was given with a 9-day broth culture but a moderate amount was demonstrated in a 10-day old peptone water culture.

Whether these two organisms would be accepted by bacteriologists as non-typical *B. coli* does not affect to any extent the points under consideration.

It will be noticed that 155 bacteriological examinations are recorded. Of these 16 were not examined for *B. coli*, and in 10 the neutral-red reactions are given, but the organism was either not looked for further or the method used failed to isolate it.

Of the 129 examinations left, in 34 *B. coli* was absent, in 93 certainly present, while in 2 a doubtful organism was isolated.

It may also be contended that the method used was a selective one and tended to pick out the *B. coli* which could reduce neutral-red and so does not give a fair measure of the distribution of this organism. To this it may be answered that almost all *B. coli* will reduce neutral-red to a greater or less degree and that in any case the negative results were also brushed and examined.

CONCLUSIONS.

1. In estimating the significance of *B. coli* in a sample of water the particular kind of water must be carefully considered, also the exact part of the system from which the sample is taken.

2. The number of *B. coli* present is an essential factor, but arbitrary standards of the number of this organism allowable per litre are of but little value and are fraught with considerable possibilities of error unless the particular kind of water and the local conditions are considered in every case.

3. Waters which show no *B. coli* in 50 c.c. are of a high degree

of purity, and therefore the proved absence of this organism in this amount, and still better in larger quantities, is of great value.

4. *B. coli* should be absent from at least 50 c.c. of spring water, possibly from greater amounts.

5. In upland surface waters the presence of *B. coli* in 40, 10 or even 2 or 1 c.c. means contamination, but not necessarily a contamination which it is essential to prevent. It may be from contamination with the excreta of animals grazing on the gathering areas and is by no means necessarily from sewage or other material containing specific organisms of infection. Further there is no evidence that an amount of such animal contamination sufficient to cause a considerable number of *B. coli* per litre to be present in the water is harmful.

If *B. coli* are present in numbers greater than say 500 per litre (or even in that amount) such a water is suspicious as it is rare to get so many *B. coli* in a water purely from the kind of animal contamination indicated, and further investigation is desirable. In filtered samples the number of *B. coli* is as a rule considerably reduced.

6. Chemical analysis cannot be considered a delicate method of detecting organic contamination, because it may fail with many waters in which pollution is undoubtedly taking place.

7. In surface wells *B. coli* in large numbers indicate surface or other contamination generally very undesirable if not actually dangerous. A knowledge of the position and the possibilities of contamination is very desirable in giving an opinion as to the purity of the water.

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- (12) *Ibid.* p. 104.

TABLE I.
Supply No. 1. Samples from gathering areas.

Date examined	No.	Source	No. of organisms per c.c. developing at		If <i>B. coli</i> present in ⁽¹⁾					Characters of <i>B. coli</i> isolated	Remarks
			37° C.	20° C.	1/2	1	2	10	40 c.c.		
June 3, 1901	1	Reservoir A ⁽¹⁾	2	61					-		
July 8, "	2	"	1	24				-	+	a ⁽⁴⁾	
Sept. 16, "	3	"	11	160			-	+	+	a	4 c.c. also negative
Nov. 11, "	4	"	8	218			-	+	+	e	
Jan. 31, 1902	5	"	144	1270	-	-	+	+	+	a	
Feb. 27, "	6	"	42	850	-		+	+	+	a	
Mar. 29, "	7	"	75	1040	-		-	+	+	a	
Aug. 8, 1901	8	The main stream entering Reservoir A	14	201				+	+	a	
Sept. 16, "	9	"	616	1440		+		+	+	a	
June 3, "	10	Reservoir B ⁽¹⁾	16	136				-(6 c.c.)	+	a	
July 8, "	11	"	0	14				-	+	a	
Sept. 16, "	12	"	9	198			+	+	+	a	4 c.c. also gave positive result
Nov. 11, "	13	"	36	410			+	+	+	a	
Jan. 31, 1902	14	"	36	1105	-	+	-	-	+	c	
Feb. 27, "	15	"	65	995	-		+	+	+	a	
Nov. 11, 1901	16	The largest stream entering Reservoir B ⁽²⁾	132	350		-	-	-	+	a	Rapid liquefaction of gelatine plates
"	17	A small mountain stream entering Reservoir B	4	200			-	-	+	a	4 c.c. also negative

⁽¹⁾ All samples from these reservoirs were taken about 20 yards from the shore and from beneath the surface to as far as possible avoid local contamination.

⁽²⁾ Rises 2 miles away among the hills and no possibility of contamination apart from sheep.

⁽³⁾ + = *B. coli* present; - = *B. coli* absent. ⁽⁴⁾ The letters refer to the group of *B. coli*, vide text.

TABLE II.
Supply No. 1. Samples from storage reservoirs.

Date examined	No.	Source	No. of organisms per c.c. developing at		If <i>B. coli</i> present in					Characters of <i>B. coli</i> isolated	Remarks
			37° C.	20° C.	1/2	1	2	10	40 c.c.		
June 13, 1901	1	Reservoir C ⁽¹⁾	37	282					+	a	
July 29, "	2	"	7	170				-	+	a	
Sept. 12, "	3	"	5	170				-	-		(2)
Nov. 29, "	4	"	5	118				+	+		
Jan. 29, 1902	5	"	3	132				+	+	a	
Feb. 26, "	6	"	3	94				-	+	a	
June 13, 1901	7	D ⁽¹⁾	33	274					+	a	
July 29, "	8	"	3	152				+	+	a	
Sept. 12, "	9	"	48	268				+	+	a	
Jan. 29, 1902	10	"	3	260			+	+	+		(2)
Feb. 26, "	11	"	23	156	-		+	-	+		(2)

⁽¹⁾ Collected near to outlet from reservoir. This applies to all the samples in this table.

⁽²⁾ Positive neutral-red reaction but not examined further for *B. coli*.

TABLE III.
Supply No. 1. Samples from filter beds and service taps.

Date examined	No.	Source	No. of organisms per c.c. developing at		If <i>B. coli</i> present in				Characters of <i>B. coli</i> isolated	Remarks
			37° C.	20° C.	1½	2	10	40 c.c.		
June 11, 1901	1	E filter beds. Filtered water	0	22				-	a	<i>B. coli</i> also found in the sand of the filter beds
July 29, "	2	" " " " " "	1	39		-		+		
Sept. 25, "	3	E filter beds. Unfiltered water	2	331				-	(1)	
" " "	4	" " " " " "	1	103				+	a	
Oct. 10, "	5	" " " " " "	4	115				+	c	5 c.c. also + Rapid liquefaction of gelatine plates
" " "	6	" " " " " "	9	80		-		-		
Nov. 29, "	7	" " " " " "	1	42		-		+	a	
July 29, "	8	F filter beds. Filtered water	2	38		+		+	a	
Sept. 25, "	9	" " " " " "	1	108		+		+	a	<i>B. coli</i> isolated from 100 c.c.
Jan. 28, 1902	10	" " " " " "	31	6400		+		+	(1)	
Feb. 26, "	11	Filtered water from a service tap (2)	252	500		-		+	a	
Sept. 18, 1901	12	" " " " " "	0	30		-		+	c	
Oct. 1, "	13	" " " " " "	1	34		-		+	a	
Jan. 3, 1902	14	" " " " " "	9	290		-		-	a	
July 2, 1901	15	Filtered water. Service tap in another town supplied	1	188		-		+	a	
Oct. 7, "	16	" " " " " "	12	510		-		+	a	

(1) Positive neutral-red reaction but *B. coli* not isolated.

(2) Beds and tanks disturbed by making of fresh filter bed. About 2 Bismark-brown *Claudiothrix* per c.c. also present.

(3) Water allowed to run for 10 mins. before collection. Personally collected.

TABLE IV.
Supply No. 1. *Chemical Analyses. (Summary of some analyses.)*

Date examined	No.	Source	Corresponding bacteriological examination	Appearance in 2 ft. tube and reaction	In parts per 100,000						Sediment	Remarks
					Total hardness	Chlorine	Free ammonia	Albuminoid ammonia	Nitrates	Nitrites	Phosphates	
1900												
Dec. 17	1	Reservoir A		yellow, clear, alkaline	2.3	0.7	0.004	0.0102	Nil	Nil	Nil	Fair amount. Mainly vegetable debris
" 17	2	" B		" "	2.4	0.7	0.0022	0.0099	"	"	"	"
" 17	3	Small stream taking drainage of house near Reservoir A		yellow, turbid, alkaline	2.2	0.75	0.0035	0.0118	"	"	"	Considerable. Sand & vegetable debris
" 5	4	Reservoir C		yellow-green, alk.	4.1	0.75	0.0024	0.0108	"	"	"	Small amount. Vegetable tissue
" 5	5	" D		" "	4.3	0.8	0.0022	0.0128	"	"	"	"
" 6	6	" E filter beds. Filtered water		" "	4.3	0.8	0.0018	0.0062	"	"	"	Practically nil
" 6	7	" F "		" "	3.7	0.75	0.0022	0.0096	"	"	"	"
1901												
April 3	8	Reservoir A		yellow, alkaline	2.5	1.0	0.0022	0.0124	"	"	"	Fair amt. Veg. debris, sand, animalculae, etc.
" 3	9	" B		" "	2.4	1.0	0.0024	0.0134	"	"	"	"
" 8	10	" C		" "	3.0	1.0	0.0036	0.0134	"	"	"	"
" 8	11	" D		" "	3.1	1.0	0.0024	0.0142	"	"	"	"
" 8	12	" E filter beds. Filtered water		" "	3.3	1.0	0.0032	0.0104	"	"	"	Very little. Few animalculae
" 8	13	" F "		" "	3.6	1.1	0.0018	0.0076	"	"	"	"
1902												
Jan. 10	14	Reservoir A		yellow-green, alkaline, turbid	2.8	0.8	0.0031	0.0086	"	"	"	Very slight
" 10	15	" B		" "	3.2	0.9	0.0032	0.0082	"	"	"	Traces only
" 9	16	" C		yellow, alkaline	3.7	0.9	0.0026	0.0076	"	"	"	Fair amt. Veg. debris
" 9	17	" D		" "	3.2	0.9	0.0032	0.008	"	"	"	Practically nil
" 9	18	" E filter beds. Filtered water		yellow-green, alk.	3.5	0.9	0.0024	0.0084	"	"	"	"
" 9	19	" F "		" "	3.2	0.9	0.0018	0.0084	"	"	"	"

TABLE V.
Supply No. 2. Bacteriological Examinations.

Date examined	No.	Source	No. of organisms per c.c. developing at		If <i>B. coli</i> present in				Characters of <i>B. coli</i> isolated	Remarks
			37° C.	20° C.	1/2	2	10	40 c.c.		
Jan. 25, 1901	1	Tap in one of the towns supplied	49	173						Not examined for <i>B. coli</i>
May 17, "	2	" " "	4	138					c	"
July 16, "	3	" " "	192	370					a	"
Oct. 9, "	4	Water just before filtration	41	218		+	+	+	a	Collected from covered tank which receives the filtered water
" "	5	Water filtered through quartz filters	48	120		+	+	+	d	Filtered through quartz filters
Oct. " 30, "	6	Water filtered through sand filters	7	104		-	+	+	a	Taken near outlet
Dec. 9, "	7	Tap in one of the towns supplied	7	270						
" "	8	The main reservoir	204	1220		-	+	+	a	
" "	9	Marsh water	—	about 1500		+	+	+	a	
" "	10	Stream entering river	20	320		-	-	-	a	5 c.c. — also
" "	11	River between the two reservoirs	—	—		+	+	+	a	
" "	12	Stream entering river	27	144		+	+	+	a	
" "	13	" "	3	330		-	-	-	a	
" "	14	Just before quartz filtration	350	1250		-	+	+	a	

TABLE VI.
Supplies Nos. 2, 4 and 6. Chemical Analyses.

Date examined	No.	Source	Corresponding bacteriological examination	Appearance in 2 ft. tube and reaction	In parts per 100,000						Sediment	Remarks		
					Total hardness	Chlorine	Free ammonia	Albuminoid ammonia	Nitrates	Nitrites			Phosphates	
1901	1	<i>Supply No. 2</i> Town service tap Just before filtration	—	yellow-green, alk.	2.6	1.0	0.0016	0.007	Nil	Nil	Nil	Very slight Fair amt. Veg. debris & numerous animalculae Nil Nil	Unsatisfactory degree of purity	
	2		4	" "	4.1	0.9	0.0048	0.0092	"	"	"			
	3		6	" "	5.5	0.9	0.0024	0.005	"	"	"			
	4		7	" "	8.9	1.1	0.0016	0.0042	"	"	"			
1901	1	<i>Supply No. 4</i> Storage reservoir	1	yellow, neutral	3.2	1.6	0.0042	0.0056	Nil	Nil	Nil	Small. Veg. debris and numerous animalculae Considerable amount. Mainly vegetable Nil Nil	Total solids = 7.3 Marked evidence of contamination. Possibly due to a local cause	
	2		" "	3.6	1.5	0.0512	0.0114	"	"	trace	"			
	3		4	yellow-green, alk.	4	1.6	0.0016	0.006	"	"	"			"
	4		7	yellow-green, neutral	3.5	1.6	0.002	0.0062	trace	"	"			"
1901 Mar. 16	1	<i>Supply No. 6</i> Service tap	2	yellow-green, neutral	3	1.05	0.0024	0.0044	Nil	Nil	Nil	Total solids = 4.8		
	2		4	yellow-green, alk.	3.7	1.0	0.0014	0.004	"	"	Nil			
	3		5	" "	4.7	1.0	0.0014	0.0044	"	"	Nil			

No Chemical Analyses available for Supplies 3 and 5.

TABLE VII.
Supply No. 3. *Bacteriological Examinations.*

Date examined	No.	Source	No. of organisms per c.c. developing at		If <i>B. coli</i> present in					Characters of <i>B. coli</i> isolated	Remarks
			37° C.	20° C.	1/10	1/2	2	10	40 c.c.		
Jan. 13, 1902	1	River on one side of the gauge	4	226	-	-	-	+	+	a	
" "	2	River on the other side of gauge	3	218	-	-	-	-	+	a	
" "	3	Spring running into river	2	65	-	-	+	+	+	c	
" "	4	River; top of proposed reservoir	2	188	-	-	-	-	+	a	
" "	5	Spring at upper part of area	4	458	-	-	-	-	-		
" "	6	Another spring at upper part of area	2	30	-	-	-	-	-		

Note. Samples were collected about 18 hrs. before they were examined. They were not packed in ice. The weather was very cold throughout and the samples were kept outside all night (temperature below 0° C. all the time), so that though possibly some numerical multiplication took place it was probably slight, while the number of *B. coli* present, if altered at all, would presumably be reduced.

TABLE VIII.
Supplies Nos. 4, 5 and 6. Bacteriological Examinations.

Date examined	No.	Source	No. of organisms per c.c. developing at		If <i>E. coli</i> present in				Characters of <i>B. coli</i> isolated	Remarks
			37° C.	20° C.	1/2	2	10	40 c.c.		
Jan. 12, 1901	1	Supply No. 4 Storage reservoir Service tap in the town " " " " " " " " Reservoir Main inflowing stream to reservoir Service tap in the town	15	282					c	(1)
June 21, "	2		9	150			+(6cc.)	+		
July 12, "	3		24	128			+	+		
Nov. 14, "	4		27	270		+	+	+	a	
" 28, "	5		4	176		+	+	+	a	
" " "	6		5	124		-	-	+	c	Collected 250 yards above reservoir (2)
March 6, 1902	7		2	165		-	+	+		
June 14, 1901	1	Supply No. 5 Service tap in the town (filtered) " " " " " " " "	2	102						
Nov. 21, "	2		11	352			-(5 c.c.)	-	a	Had been previously a considerable scarcity of water
Feb. 18, "	3		9	730	-	+	+	+	a	
Feb. 11, 1901	1	Supply No. 6 Service tap (filtered) " " " " " " " " " " " "	10	71						
March 16, "	2		4	73						
July 2, "	3		7	88			-	+	a	
Jan. 21, 1902	4		3	80		-	-	+	c	
March 25, "	5		0	40	-	-	-	+	a	

(1) Positive neutral-red reaction with the 10 and 40 c.c. but not examined for *B. coli*.

(2) *B. coli* could not be found. Probably present but missed as no other neutral-red reacting organism found.

TABLE X.
Supplies Nos. 7, 8 and 9. Chemical Analyses.

Date examined	No.	Source	Corresponding bacteriological examination	Appearance in 2 ft. tube and reaction	In parts per 100,000						Sediment	Remarks
					Total hardness	Chlorine	Free ammonia	Albuminoid ammonia	Nitrites	Phosphates		
Supply No. 7												
1901	1	Service tap in the town	1	yellow, neutral	2.3	1.65	0.002	0.0132	Nil	Nil	Fair amount. Chiefly vegetable matter	
	2	" " "	3	" " "	3.4	1.6	0.006	0.0148	"	"	Small amount	
1902	3	" " "	4	" " "	3.3	1.6	0.0012	0.008	"	"	Practically nil	
	4	Storage reservoir	5	" " "	3.3	1.65	0.005	0.0158	"	"	Fair amt. Veg. cells & debris. Numerous animalculae	
	5	Service tap in the town	6	yellow-green, neut.	3.3	1.7	0.0016	0.0082	"	"	Small amount	
Supply No. 8												
1901	1	Service tank		yellow-green, faintly alkaline	3.0	1.5	0.0052	0.0076	faint trace	Nil	Fair amount. Mainly vegetable debris	
	2	Tap	1	" " "	2.7	1.6	0.0012	0.0044	Nil	"	Traces only	
1902												
	3	From service tank	2	yellow-green, neut.	3	1.7	0.004	0.0132	faint traces	"	Fair amount. Veg. debris & numerous animalculae	
Supply No. 9												
1901	1	Filtered Reser. C water	1	yellow-green, alk.	5.3	1.0	0.0024	0.0062	Nil	Nil	Nil	
	2	" " "	2	yellow, neutral	3.4	1.0	0.0034	0.0174	"	"	Fair amount. Veg. cells & debris	
	3	" " " A & B "	3	" " "	4	0.9	0.0014	0.0146	"	"	Small amount	
1902												
	4	Same as No. 4 bact. sample	4	yellow-green, alk.	4	1.0	0.002	0.0084	traces	"	Fair amount	
	5	Reservoir C overflow	5	" " "	4	1.0	0.0036	0.0108	"	"	Considerable amt. Animalculae extremely numerous	
	6	Reservoir C just after filtration	7	" " "	4	1.0	0.0014	0.0098	"	"	Small amount only	

TABLE XI.
Supplies Nos. 10, 11, 12, 13 and 14. Bacteriological Examinations.

Date examined	No.	Source	No. of organisms per c.c. developing at		If <i>B. coli</i> present in				Characters of <i>B. coli</i> isolated	Remarks	
			37° C.	20° C.	1/2	2	10	40 c.c.			
<i>Supply No. 10</i>											
Jan. 25, 1901	1	Tap in town supplied	1	45							
May 10, "	2	" "	1	126			-	-			
Nov. 1, "	3	" "	0	18		-	-	-			
Jan. 31, 1902	4	" "	3	65		-	-	-			
<i>Supply No. 11</i>											
Feb. 20, 1901	1	Supply tap	2	62							
Sept. 4, "	2	" "	3	14		-	-	-			
Jan. 6, 1902	3	" "	0	20		-	-	-			
Mar. 17, "	4	" "	1	82		+	-	-			<i>B. coli</i> present in Winchester quart of the water (1)
<i>Supply No. 12</i>											
Feb. 20, 1901	1	Tap in town supplied	1	46							
Nov. 20, "	2	" "	0	70		-	+	+	a		
Feb. 18, 1902	3	A tap in another part of the district	7	1190	-	-	-	-	c		
<i>Supply No. 13</i>											
Feb. 5, 1902	1	Tap	1	85		-	-	-			
<i>Supply No. 14</i>											
Feb. 6, 1902	1	Tap in town supplied	1	27		-	-	-			

(1) A positive neutral-red reaction with the 2 c.c. but not examined further. Probably an accidental contamination.

TABLE XII.

Supplies Nos. 10, 11, 12, 13 and 14. Chemical Analyses.

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Date examined	No.	Source	Corresponding bacteriological examination	Appearance in 2 ft. tube and reaction	In parts per 100,000						Sediment	Remarks
					Total hardness	Chlorine	Free ammonia	Albuminoid ammonia	Nitrites	Phosphates		
Supply No. 10												
1901 Jan. 25	1	Tap in town	1	almost colourless, neutral	3.5	1.0	0.0014	0.003	Nil	Nil	Nil	Total solids = 5.2 Very high degree of organic purity Total solids = 4.9
May 10	2	"	2	faint yellow-green, faintly alk.	3.4	1.2	0.001	0.0046	"	"	Small amount	
Nov. 1 1902	3	"	3	yellow-green, neut.	3.0	1.3	0.0014	0.0042	"	"	Nil	
Jan. 31	4	"	4	" alk.	3.8	1.3	0.001	0.0024	traces	"	Traces only	
Supply No. 11												
1901 Feb. 20	1	Service tap in town	1	yellow-green, neut.	2	1.0	0.0018	0.0038	traces	Nil	Slight	Total solids = 8.8 All the samples show a high degree of organic purity
Sept. 4 1902	2	" "	2	" "	3.3	1.05	0.0014	0.0038	Nil	"	Practically nil	
Jan. 6 Mar. 17	3 4	" "	3 4	" "	2.5 3	1.0 1.05	0.0014 0.001	0.003 0.003	" traces	" "	" "	
Supply No. 12												
1901 Feb. 20	1	Tap in town supplied	1	yellow-green, alk.	3.2	1.1	0.002	0.0042	Nil	Nil	Practically nil	
Nov. 20 1902	2	" "	2	" "	6	1.1	0.001	0.0026	"	"	A small amount	
Feb. 18	3	Tap in different district	3	" "	6.7	1.0	0.001	0.0032	traces	"	Considerable amt. Veg. debris and a few animalculae	
Supply No. 13												
1900 Dec. 14	1	Tap	—	yellow-green, neut.	3.9	2.2	0.0016	0.0022	Nil	Nil	Small amount	A high degree of organic purity
Supply No. 14												
1902 Feb. 6	1	Tap in town supplied	1	yellow-green, neut.	4	1.3	0.0016	0.0024	faint traces	Nil	Nil	" " "

TABLE XIII.
Supplies Nos. 15, 16 and 17. Bacteriological Examinations.

Date examined	No.	Source	No. of organisms per c.c. developing at		1/2	If <i>B. coli</i> present in				Characters of <i>B. coli</i> isolated	Remarks
			37° C.	20° C.		2	10	40 c.c.			
<i>Supply No. 15</i>											
Oct. 31, 1901	1	The covered reservoir	2	26		-	-	-	c	(1)	
Nov. 14, "	2	" "	2	24		-	-	+			
" 28, "	3	Tap in town supplied	1	60		-	-	+			
Mar. 20, 1902	4	The covered reservoir	10	64	-	-	-	-			
" "	5	Tap in town supplied	1	48	-	-	-	-			
<i>Supply No. 16</i>											
July 8, 1901	1	Tap in town supplied	2	206		-	-	-			
Feb. 6, 1902	2	" "	0	30		-	-	-			
<i>Isolated Samples from different sources (No. 17)</i>											
Oct. 22, 1901	1	Tap in village supplied	0	136		-	-	-			A number of springs. Water passes by pipes to covered reservoir and from thence by pipes to village a mile away, not filtered An unfiltered spring water No dwellings on hill side
" 28, "	2	" "	1	31		-	-	-			
Nov. 7, "	3	A spring issuing from hill side	0	30		-	-	-			No possibility of contamination
Mar. 20, 1902	4	A tap in village supplied ⁽²⁾	0	12		-	-	-			Spring from the sandstone
Feb. 18, "	5	A spring water	1	38	-	-	-	-			No known contamination but examined because 7 cases of enteric fever in the area supplied
April 11, "	6	The same water as No. 1	0	12		-	-	-			
Sept. 14, 1901	7	Spring on mountain side	7	45		-	-	-			

(1) Positive neutral-red reaction with 40 c.c. but *B. coli* not found.

(2) A spring which runs over a considerable amount of ground before it enters the pipe which conveys it to the houses supplied. Contamination with surface water is possible and often no doubt takes place. Said to be a scarcity in summer.

TABLE XIV.

Supplies Nos. 15, 16 and 17. Chemical Analyses.

Date examined	No.	Source	Corresponding bacteriological examination	Appearance in 2 ft. tube and reaction	In parts per 100,000						Sediment	Remarks	
					Total hardness	Chlorine	Free ammonia	Albuminoid ammonia	Ni- trates	Ni- trites			Phos- phates
<i>Supply No. 15</i>													
1902 Mar. 20	1	The covered reservoir	4	yellow-green, alk.	14.8	1.1	0.004	0.0102	traces	Nil	Nil	Traces only	Very high albuminoid ammonia for this class of water and this is unsatisfactory. This is not shown in the tap specimen
" "	2	Tap in town supplied	5	" "	15.4	1.1	0.0014	0.0036	"	"	"	"	
<i>Supply No. 16</i>													
1901 July 8	1	Tap in town supplied	1	yellow-green, alk.	7	1.45	0.0018	0.0026	traces	Nil	Nil	Practically nil	High degree of organic purity
1902 Feb. 6	2	" "	2	" "	8.8	1.4	0.0010	0.0020	"	"	"	Nil	" "
<i>Isolated Samples from different sources (No. 17)</i>													
1901 Oct. 28	1	Tap water	2	yellow-green, alk.	13	2.9	0.0012	0.0042	traces	Nil	Nil	Nil	High degree of organic purity
1902 Feb. 18	2	Spring water	5	" "	4.6	1.5	0.0014	0.0024	"	"	"	"	" "

Very high albuminoid ammonia for this class of water and this is unsatisfactory. This is not shown in the tap specimen

High degree of organic purity

" "

" "

High degree of organic purity

" "

TABLE XV.
Shallow Wells. Bacteriological Examinations.

Date examined	No.	Source	No. of organisms per c.c. developing at		If <i>B. coli</i> present in				Characters of <i>B. coli</i> isolated	Remarks
			37° C.	20° C.	1/2	2	10	40 c.c.		
<i>Isolated Samples from different sources (No. 18)</i>										
June 26, 1901	1	A shallow well	6	107			-	+	a	Gelatine plates rapidly liquefied and fig. represents only averages of the 2 day counts. (1) Same water as No. 2
July 1, "	2	Pump water. Shallow well	202	1120			-	+	a	
" 2, "	3	A shallow well	1080	10,000			+	+	a	
" 7, "	4	" "	4	254			+	+	a	
" 22, "	5	" "	140	980			+	+	a	
Oct. 22, "	6	A well water	117	2100			+	+	a	
" 30, "	7	A surface well	19	480		+	+	+	d	
Nov. 15, "	8	" "	1	56			+	+	a	
Jan. 4, 1902	9	" "	—	330		-(1 c.c.)	+	+	c	
" 23, "	10	" "	82	220		-(1 c.c.)	+	+	a	
Feb. 8, "	11	A well water	1370	1800	-	+	+	+	a	
" 5, "	12	A surface well	38	2400		+	+	+	b	
" 12, "	13	" "	130	1920	-	-	+	+		
Sept. 28, 1901	14	Pump water. Shallow well	25	104		+	+	+	a	
<i>No. 19. A shallow well</i>										
Jan. 22, 1901	1	From the pump	11	174			+	+	a	Gelatine rapidly liquefied and 20° C. count = average for 2 days
Dec. 18, "	2	" "	40	580		+	+	+	c	
Mar. 17, 1902	3	" "	520	3100	+	+	+	+		

(1) A positive neutral-red reaction with the 10 c.c. but not examined further.

TABLE XVI.
Shallow Wells. Chemical Analyses.

Date examined	No.	Source	Corresponding bacteriological examination	Appearance in 2 ft. tube and reaction	In parts per 100,000							Sediment	Remarks
					Total hardness	Chlorine	Free ammonia	Albuminoid ammonia	Nitrates	Nitrites	Phosphates		
<i>Isolated Samples from different sources (No. 18)</i>													
1901 June 26	1	A surface well	1	yellow-green, alk.	10.2	1.85	0.0016	0.0066	traces	Nil	Nil	Small amt. Veg. deb. Animalculae fairly numerous	Same water as No. 2
July 1	2	" "	2	" "	29	2.5	0.0020	0.0048	marked traces	"	"	Fair amt. Chiefly veg. deb. Animalculae fairly numerous	
1902 Jan. 4	3	" "	9	neut.	11.2	3.7	0.0032	0.0048	traces	"	"	Fair amt. Veg. deb. mainly	
" 23	4	" "	10	" alk.	26.4	2	0.0012	0.0028	marked traces	"	"	Practically nil	
Feb. 8	5	A well water	11	" "	7	2.6	0.0058	0.0076	traces	"	Nil	Considerable amt. Mainly veg. deb. A few animalculae	
1901 Sept. 28	6	A surface well	14	" "	28	2.5	0.0018	0.0048	distinct traces	"	"	Fair amt. Veg. deb. and a few animalculae	
<i>Supply No. 19</i>													
1901 Jan. 22	1	From the pump	1	yellow-green, alk.	32	2.1	0.0018	0.0052	traces	Nil	Nil	Traces only	Total solids = 37.8
Dec. 18	2	" "	2	" "	44	6.3	0.0024	0.0108	well marked	"	"	Small amount. Veg. deb. mainly	Volatile " = 6.9
1902 Mar. 17	3	" "	3	yellow, alkaline	43	5.6	0.0026	0.0130	1.2	"	traces	Small amount. Veg. deb. mainly	Permanent hardness 11.2
													Evidence of contamination

TABLE XVII.
Supply No. 20. Bacteriological Examinations.

Date examined	No.	Source	No. of organisms per c.c. developing at		If <i>B. coli</i> present in					Characters of <i>B. coli</i> isolated	Remarks
			37 °C.	20 °C.	1/10	1/2	2	10	40 c.c.		
Oct. 13, 1900	1	Tap at pumping station	72	196							
Jan. 16, 1901	2	" "	136	408						a	5 c.c. only examined and from this <i>B. coli</i> isolated.
Feb. 14, "	3	" "	266	473						a	Fatal to a guinea-pig
Mar. 22, "	4	" "	1980	2108							Not brushed or further examined for <i>B. coli</i>
" "	5	Tap in town supplied	484	1278			+		+		
May 16, "	6	Tap at pumping station	692	1780			-		-		
June 25, "	7	Water in penstock valve chamber	7	42			+		(30 c.c.)		
" "	8	Tap at pumping station	210	370				(partial)	+		
July 6, "	9	Water in penstock valve chamber	191	620			-		+	a	Collected myself and started within an hour of being collected
" "	10	Tap at pumping station	135	402					+	a	
Oct. 7, "	11	" "	5	60					+	a	
Jan. 14, 1902	12	" "	30	130		+(1 c.c.)	+	+	+	c	
" "	13	Tap in town supplied	65	392		-	+	+	+	a	
" "	14	An accessory spring water flowing into one of the reservoirs	14	94		-	-	+	+	d	
Jan. 29, "	15	Another accessory spring in district	115	1420		+	+	+	+	a	
April 2, "	16	Tap at pumping station	150	780			+	+	+	a	

(1) A positive neutral-red reaction but not examined further.

TABLE XVIII.

Supply No. 20. Chemical Analyses.

Date examined	No.	Source	Corresponding bacteriological examination	Appearance in 2 ft. tube and reaction	In parts per 100,000							Sediment	Remarks	
					Total hard- ness	Chlo- rine	Free ammonia	Albu- minoid ammonia	Ni- trates	Ni- trites	Phos- phates			
1900														
Oct. 13	1	Tap at pumping station	1	yellow-green, alk.	37	2.6	0.0020	0.0044	0.47	Nil	Nil	Practically nil	Total solids = 49.0	
1901														
Jan. 16	2	" "	2	"	36	2.5	0.0022	0.005	distinct traces	"	"	Small amount. A few animalculae	Total solids = 38	
Mar. 22	3	" "	4	"	38	2.5	0.0018	0.0088	"	"	"	Practically nil		
July 20	4	" "	"	"	38	2.9	0.0032	0.0078	"	"	"	"		
Oct. 7	5	" "	11	"	39	2.7	0.0018	0.0044	"	"	"	"		
1902														
Jan. 14	6	" "	12	"	38.6	2.7	0.0016	0.0042	"	"	"	"		
" "	7	Accessory spring	14	"	35	2.3	0.002	0.0078	Nil	"	"	Small amt. Veg. deb.		
Jan. 29	8	Another accessory spring	15	"	38.4	1.4	0.0014	0.0064	traces	"	"	Small amt. Veg. deb. and a few animalculae		
April 2	9	Tap at pumping station	16	"	38.4	2.6	0.0014	0.0018	distinct traces	"	"	Traces only		

ON THE CONSTRUCTION AND USE OF LIFE-TABLES FROM A PUBLIC HEALTH POINT OF VIEW.

By T. E. HAYWARD, M.B. (LOND.), F.R.C.S. ENG.,

Medical Officer of Health for Haydock, Lancashire.

(Concluded.)

It was not possible to complete the considerable amount of work necessary for the following Addendum in time for inclusion with the article which appeared in the last number of this *Journal*.

In order to submit the method explained in the last number of the *Journal of Hygiene* for graphically constructing the curve of $\log p_x$ values to as exact a test as possible, in the first place the data of the Life-Table for England and Wales for 1881-90 (males) have been taken, and a *complete series of $\log p_x$ values* calculated from them according to the methods of interpolation previously described (see the Articles in the two preceding numbers of the *Journal of Hygiene*), and then in the next place the values obtained by the graphic method have been taken and compared one by one with the values obtained by exact calculation. In order to save space there will only be given below the *differences of the measured from the calculated values*.

Please note that the value given for age 5 expresses the difference of the measurement of the ordinate $5\frac{1}{2}$ from the calculated value of $\log p_5$, &c.,

Age	Differences	Age	Differences	Age	Differences	Age	Differences	Age	Differences
5	+ '00007	25	± '00000	45	+ '00002	65	+ '00004	85	- '00015
6	+ '00011	26	- '00004	46	± '00000	66	+ '00005	86	- '00032
7	+ '00005	27	- '00005	47	+ '00002	67	- '00001	87	- '00001
8	+ '00003	28	- '00005	48	+ '00001	68	+ '00002	88	- '00004
9	+ '00001	29	- '00003	49	± '00000	69	+ '00003	89	+ '00002
10	+ '00003	30	+ '00002	50	+ '00001	70	+ '00002	90	- '00012
11	+ '00001	31	+ '00004	51	- '00003	71	+ '00001	91	+ '00001
12	- '00005	32	+ '00006	52	- '00003	72	+ '00003	92	- '00005
13	- '00006	33	+ '00003	53	- '00002	73	+ '00008	93	- '00001
14	- '00002	34	+ '00002	54	- '00004	74	+ '00006	94	+ '00008
15	- '00001	35	+ '00001	55	- '00004	75	- '00002	95	+ '00016
16	+ '00003	36	- '00001	56	- '00007	76	- '00004	96	± '00000
17	+ '00003	37	- '00001	57	- '00009	77	- '00001	97	+ '00004
18	± '00000	38	+ '00001	58	- '00008	78	- '00014	98	+ '00013
19	+ '00002	39	- '00003	59	- '00004	79	- '00009	99	- '00016
20	- '00002	40	± '00000	60	+ '00002	80	- '00005	100	- '00007
21	- '00003	41	± '00000	61	+ '00005	81	- '00007	101	+ '00005
22	- '00001	42	+ '00001	62	+ '00009	82	- '00003	102	+ '00006
23	- '00004	43	± '00000	63	+ '00007	83	± '00000	103	- '00047
24	- '00003	44	- '00001	64	+ '00001	84	+ '00013		

[A somewhat similar comparison, almost equally exact, has also been made from the data of the Life-Table for Selected Healthy Districts (males)].

The actual work of drawing and measuring the curves has been done for the writer by a mining surveyor who had no knowledge of what the calculated values were, and who had given to him as data the $\log p_x$ values $4\frac{1}{2}$, 5, 10, 15, 20, 25, 30...105, and also $67\frac{1}{2}$, $72\frac{1}{2}$, $77\frac{1}{2}$, $82\frac{1}{2}$, $87\frac{1}{2}$, $92\frac{1}{2}$, $97\frac{1}{2}$, $102\frac{1}{2}$, obtained by calculations as described in the Articles already alluded to.

The curve has been drawn in three sections:—

Section I, from $4\frac{1}{2}$ to 75. Vertical scale $\frac{1}{8}'' = \cdot 00050$, horizontal scale $\frac{1}{8}'' = \frac{1}{2}$ year. This has been used for measuring from $5\frac{1}{2}$ to $64\frac{1}{2}$ inclusive.

Section II, from 55 to 95. Vertical scale $\frac{1}{8}'' = \cdot 00100$, horizontal scale, $\frac{1}{8}'' = \frac{1}{4}$ year. This has been used for measuring from $65\frac{1}{2}$ to $89\frac{1}{2}$ inclusive.

Section III, from 85 to 105. Vertical scale $\frac{1}{8}'' = \cdot 00250$, horizontal scale, $\frac{1}{8}'' = \frac{1}{8}$ year. This has been used for measuring from $90\frac{1}{2}$ to $103\frac{1}{2}$ inclusive.

The measured values have been taken as returned to the writer *without revision*.

A complete Life-Table worked out from them would not differ very sensibly from one constructed from the calculated series of $\log p_x$ values.

Even the largest differences are insignificant compared with the differences which may be obtained between the results of different systems of analytical interpolation.

Occasion may be taken to note:

(1) The formula which has been given for $\log p'_{20}$ (or u_{20}), see the last number of this *Journal*, p. 211, has inadvertently not been reduced to its lowest terms; as all the co-efficients are divisible by 3, it should be

$$u_{20} = \frac{(35u_5 + 420u_{15} + 210u_{25} + 3u_{45}) - (192u_{10} + 28u_{35})}{448}.$$

(2) A more accurate formula for $\log p'_{30}$ (or u_{30}) is

$$u_{30} = \frac{(320u_{10} + 1050u_{25} + 630u_{35} + 7u_{55}) - (63u_5 + 525u_{15} + 75u_{45})}{1344}.$$

ENTERIC FEVER AND SEWAGE DISPOSAL IN TROPICAL COUNTRIES.

(One Map.)

By MAJOR A. R. ALDRIDGE, R.A.M.C.,

Naini Tal, N.W.P., India.

THAT many epidemics of enteric fever have been caused by contamination of central water-supplies is beyond dispute, but evidence is accumulating that makes it difficult to attribute its widespread prevalence in endemic form in India and elsewhere to this cause. It is necessary, however, to guard against assuming that therefore the disease is not water-borne. There are in India innumerable chances of water, when stored for domestic use, being contaminated, besides the same possibility in the case of food, feeding utensils, and cloths used for cleaning them. And when it is claimed that dust or flies play an important rôle in its dissemination, it is not necessary to assume that the bacillus is taken into the mouth or respiratory passages directly, but rather that it is conveyed to water, &c., by means of dust or flies.

There seems, indeed, a danger of constructing two apparently opposed theories, where in fact the one is actually the complement of the other.

In Indian cantonments sudden, severe epidemics, such as are to be expected as the result of contaminated central water-supplies, account for but a small proportion of the cases of enteric fever: and pipe water-supplies have not produced the improvement that was expected, although in many cases their construction is such as to make it difficult to conceive the possibility of repeated pollution. Nor has the boiling of drinking water, which has been carried out for several years in many stations, apparently produced any reduction. It is noticeable, however, that in almost all cases the boiled water has been stored in detail in barracks, this being necessary so as to allow it to cool.

The following table shows the prevalence of enteric fever among British troops in all stations in India with an average strength of over 1,000.

Average annual admission rate per 1,000. 1889-98¹.

Agra	48·6	Rawl Pindi	28·6
Bareilly	40·6	Secunderabad	25·2
Lucknow	40·2	Bangalore	20·3
Chakrata	36·8	Poona	18·1
Ranikhet	36·5	Kamptee	12·2
Meerut	35·6	Rangoon	9·6
Mhow	35·1	Wellington	8·8
Allahabad	34·5	Mandalay	6·1
Sialkote	33·0	Calcutta	5·4
Umballa	31·1	Aden	4·9
Quetta	30·7	Bombay (Colaba)	4·2
Peshawar	30·1	Belgaum	2·9

Of these 24 stations the 13 having the highest admission rates are geographically very distinctly separated from those having low rates; all being situated in, or closely bordering on the dry, dusty, alluvial plain of Upper India. The climate of this tract is for a great part of the year excessively dry and dusty. On the other hand, of the 11 stations having a relatively low rate four are situated on or near the coast, with a damp climate throughout the year; while the remainder are situated on rocky soils of volcanic origin and are relatively free from dust.

Further, in the first group the season of greatest prevalence is during April and May, the driest and dustiest months of the year.

The following table shows the total number of cases in these 13 stations during the period 1891-1900.

Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
421	193	439	884	843	598	583	640	620	467	538	599

In the other group there is an exacerbation during the rainy season, in August, which in some cases is greater than that in the spring, as the following table shows:

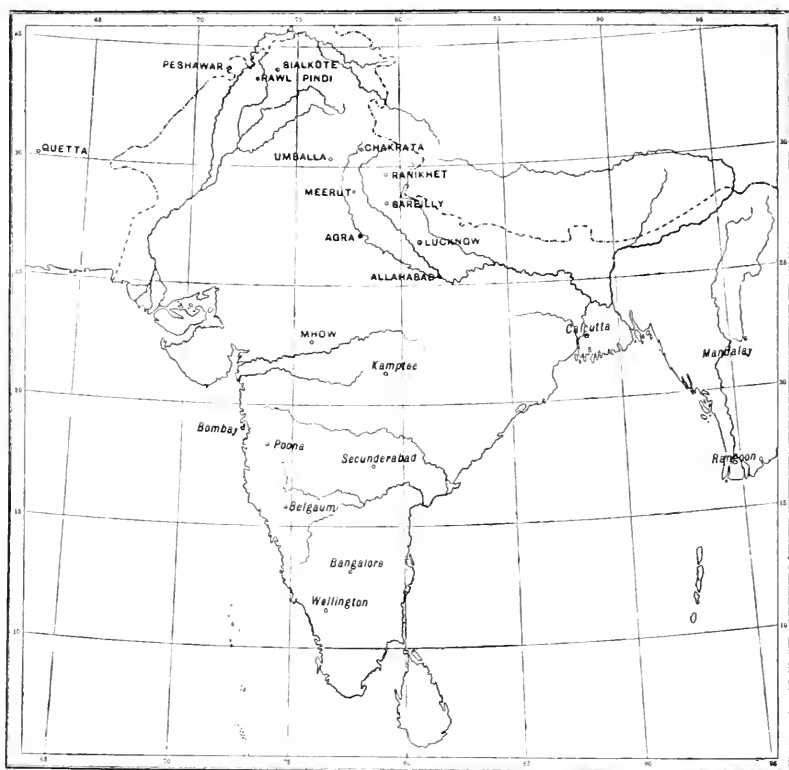
Total admissions in group II., 1891-1900.

Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
93	124	148	128	112	114	219	296	229	144	118	84

No peculiarity of water-supplies will account for these differences, for well and pipe supplies are to be found in both groups. As regards sewage disposal, the dry earth system of latrines is in force in all; and

¹ Annual Report of the Sanitary Commissioner with the Government of India, 1899, p. 51.

with only one or two exceptions a trench system (either shallow or deep) of disposal.



All places in capitals have an admission rate over 28 per 1000.

All places in italics have an admission rate under 26 per 1000.

The latrines and urinals are not provided with impervious floors, and all spillage, which,—from their construction—in the case of urinals is frequent, soaks into what is in most stations a dry, powdery earth.

It is a matter of common observation that water stored in such dry, dusty places will in a very short time be covered with a scum of dust, even though the receptacle be covered.

The same must happen with food and utensils: while the habit of Indian cooks of “cleaning” metal vessels,—in spite of all protests—with earth, may also be a means of this dust reaching water and food. That some of this dust comes from the floors of the latrines and urinals, scattered as they are all over barracks, often at no great distance from cook-houses and stored water, can hardly be doubted.

Similarly in many stations the trenching grounds are sufficiently close to barracks for the sandy soil of which they are composed to be easily carried by the wind, and particularly by dust-storms, which are of frequent occurrence in many of these stations during the dry season.

Recent researches tend to show that the vitality of *B. typhosus* in water and soil is not great; in fact, as Horton-Smith¹ has pointed out there is so far no proof that the bacillus can multiply outside the human body. Such an assumption is, however, not necessary to account for its frequent presence in polluted soils, for it has been fully established that persons recovered from an attack of the disease may for months and years continue to disseminate the bacillus in the faeces, and more particularly the urine.

These researches seem to indicate that when enteric fever is almost continuously present there must be repeated infections from fresh evacuations, which can be explained in the way suggested; but such can hardly be conceived in the case of well-protected pipe supplies. Numerous instances could be cited in support of this view.

Two epidemics in Cherat², one of which was investigated by the writer, showed a marked tendency for the cases to occur in groups, with an interval closely corresponding to the incubation period of the disease, suggesting that each group was infected from the former. Thus, one barrack furnished its first case on 27th May, the second 14 days later, a third 15 days later, and after another interval of 10 days six cases between 5th and 20th July. Another barrack had its first case on 19th June, two following on 25th and 28th; after an interval of 16 days five cases between 14th and 23rd July, and one more case after another interval of 11 days. A third barrack, commencing on 7th July, had five cases in five days, and 14 days later two more. Both epidemics attacked chiefly (one almost exclusively) the occupants of barracks at one end of the station, close to the incinerator, where faecal matter was continually standing in heaps, mixed with dry refuse to partially dry before it could be put into the incinerator. The water was derived from protected springs, four miles distant from the station. The springs were well away from any apparent source of contamination. The water was however stored in barracks in loosely covered vessels, and dust-storms were of frequent occurrence. May not each of these groups of cases

¹ Goulstonian Lectures for 1900, *Lancet*, Vol. i. p. 826.

² Annual Report of the Sanitary Commissioner with the Government of India, 1899; and Report by Major A. M. Davies, R.A.M.C., "On Sanitary Investigations and Bacteriological examinations at Cherat," June, 1898.

have been infected by this means from the evacuations of the former cases, passed during the early stages of the disease, before admission to hospital?

Recently enteric fever was very prevalent in a station with a pipe water-supply derived from protected wells, and in which no possible source of contamination could be found. I failed to find in the water either *B. typhosus* or any organism indicating sewage contamination. Nor could any connection be traced between the cases and any particular supply of food, dairy produce, or mineral water, nor with any particular place of residence or resort. It was found that the clothes, including kitchen cloths, were being washed at a place very close to the filth trenches, so that dust from the trenches was being blown in clouds over them. I exposed a sterilised cloth to this dust, and from "washings" from this isolated *B. coli*¹ and a bacillus giving the typical reaction in milk of the *B. enteritidis sporogenes*.

The following experiments were undertaken with a view of testing how far the bacterial contamination of water by means of dust could be traced.

In order first to ascertain the bacterial constituents of soils which might be expected to be polluted, 1 c.c. of soil was mixed with 100 c.c. of sterile water and this water examined.

Streptococci were searched for on surface agar plates, and suspected colonies sub-cultured in broth.

For the colon group, the water was added to 0.5% carbolic broth, incubated for 24 hours at 37° C. and then plated on glucose-litmus agar and carbolic agar.

B. enteritidis sporogenes was searched for by the usual reaction in milk.

Water No. 3 was from a filtered pipe-supply, frequently examined bacteriologically and containing about 30 colonies per c.c., none resembling *B. coli*.

The tank No. 4 was filled by a pump and pipe from a covered well. The water of this well, examined at the same time, contained 185 colonies per c.c. and did not show the presence of *B. enteritidis sporogenes*.

Cladothrix and *B. mycoides* are not commonly found in good waters²,

¹ This bacillus was typical as regards colonies and microscopical appearances. Gas was produced in glucose-agar in 24 hrs. Indol in 4 days. Milk not coagulated in 3 days.

² Houston, "Report on the Chemical and Bacteriological Examinations of Soil Washings." 28th Annual Report of the Medical Officer to the Local Government Board, pp. 453 and 455.

	Number of colonies per c.c.	Streptococci	<i>B. Coli</i>	<i>B. enteritidis sporogenes</i>
1. Soil from floor of latrine	26400 <i>B. mycoides</i> very numerous; <i>Cladothrix</i>	.01 c.c. Present Broth turbid, stringy deposit, pellicle On agar colonies granular, no chains at edges Cocci in short chains or some groups	1. c.c. Considerable number of coliform colonies Milk coagulated in 48 hrs. Gas in 24 hrs. Indol in 4 days	1. c.c. Not present
2. Soil close to a latrine	18500 <i>B. mycoides</i> ; <i>Cladothrix</i>	.01 c.c. None	2. c.c. Coliform Colonies not numerous Milk coagulated in 4 days No gas Indol in 6 days Neutral red decolorised	1. c.c. Present Reaction in 24 hrs.
3. Water stored in an uncovered 'chattie' in a barrack-room	283		1. c.c. Considerable number of coliform colonies Milk coagulated in 4 days Indol in 5 days No gas	
4. Water from iron tank, with loosely fitting cover; 40 yds. from a latrine	620	.5 c.c. None	5. c.c. None	1. c.c. Present Reaction in 24 hrs.
5. Sterile water exposed in bottle 10 yds. from a latrine for 1 hr. on windy day	1290 <i>Cladothrix</i> (50 per c.c.) <i>B. mycoides</i> very numerous	.1 c.c. None	5. c.c. None	1. c.c. None

though I have occasionally found them in small numbers in water from uncovered wells, in which cases also they may have been derived from dust.

These observations indicate that water may be contaminated by various bacteria carried by dust. Since *B. typhosus* has on several occasions been isolated from the soil, and it having been proved that it can withstand a considerable amount of drying¹, it can hardly be doubted that the bacillus may be conveyed by dust.

In accounting for the almost universal prevalence of *B. typhosus* in Indian cantonments the probability of infection from native sources must not be overlooked. There can be little doubt that the disease is much more common among natives than has hitherto been supposed. Thus, Captain G. Lamb, I.M.S., has recently published² a series of 11 cases, the diagnosis having been confirmed in 9 by the sedimentation test, in 2 by post-mortem appearances. Other observers³ have also recorded considerable numbers of cases, in many of which the diagnosis has been similarly confirmed.

The histories of modern campaigns still further confirm these views. All recent British campaigns, with the exception of Ashanti, in which enteric fever was absent, have been in dusty climates, and in all enteric fever has been very prevalent. The conditions of active service add to the facilities for the dispersal by dust and flies of excremental matter, dry methods of disposal being always used. At the Modder River, S. Africa, the soil was "trampled and pulverized by thousands of feet to an impalpable powder. This mixed with excreta was wafted in dense clouds." The men "urinated and defaecated in the neighbourhood of the tents⁴." No doubt in some cases central water-supplies have been infected, but apparently not in all. In the Spanish-American war at Jacksonville, Lexington, and Knoxville the troops used water from the same source as did the civil population, yet while the troops suffered severely the civil population practically remained exempt⁵. In Egypt, in 1885, certain of the troops supplied with distilled drinking-water suffered severely⁶.

¹ Macé, *Traité de Bactériologie*, p. 710.

² Lamb, G., from Plague Research Laboratory, Bombay. "Typhoid fever in natives of India, its diagnosis by means of the serum sedimentation reaction."

³ Buchanan, A., *Indian Med. Gazette*, 1899, pp. 336, 403, 446; 1900, pp. 53, 174. Rogers, L. *Ibid.* 1902.

⁴ Ryerson, G. S. (Lieut.-Col., Canadian Army Medical Staff). Address to Toronto Clinical Society, Oct. 1900.

⁵ Munson, *Military Hygiene*, p. 681.

⁶ Army Med. Department Report, 1885.

In European towns, too, the decline in enteric fever has been noticed to follow more closely on the substitution of sewers for various dry methods of removal than upon the improvement of water-supplies.

Death-rate per 1000 from Enteric Fever¹.

	Before sewerage	After sewerage
Frankfurt	·87	·24
Dantzic	·90	·18
Munich	2·42	·17

In the Leicester epidemic of 1894 one street showed five times as many infected houses among those using the tub-system as among those having water-closets and sewers. At Newcastle cases were twice as numerous in houses with the dry-earth system as in those with water-closets: and in Birmingham the incidence was one and a half times greater with pails than with water-closets².

I have attempted to show that in combating enteric fever in tropical places it is not sufficient to obtain a water pure at its source, nor even to purify a doubtful water; but that the chances of its contamination later are as great,—in many cases greater—than at its source. I believe that these possibilities have not received the attention they deserve. What means, then, should be adopted to meet this contingency?

(1) Avoidance as far as possible of all storage of water near habitations where, from the proximity of dry-earth latrines and filth trenches, it may be polluted.

Boiling, except as a temporary measure, where there is definite reason to suspect contamination of the supply is likely to add to rather than lessen the risk, on account of the storage necessary for cooling. Boiling and cooling can only safely be carried out in a special apparatus, such for instance as the Waterhouse-Forbes, which seems to have proved a success in America.

(2) Latrines and urinals should be situated as far as practicable from kitchens and from stored water. At present it is only too common to see them within 30 or 40 yards of uncovered wells, water tanks and kitchens. They should have impervious floors, from which spillage can be removed by washing, so that it may not contaminate the dust.

(3) All food, feeding utensils and everything used in their preparation (including the washing of cloths, &c.,) should be protected from dust and flies.

(4) It can hardly be expected, however, that these means alone

¹ Oldright, quoted by Munson, *loc. cit.* p. 541.

² Moore, quoted by Munson, *loc. cit.* p. 538.

will be enough, and a thorough trial, at some place where enteric fever has for many years been excessive, of water-carriage of sewage, combined with a method of disposal that will avoid dust dissemination seems more than justified. For the carriage of sewage, water-closets and sewers would be the most satisfactory method: though trough closets and removal by pumping into iron tanks on wheels might, for economic reasons, have to be substituted in some cases, it can only be considered as distinctly inferior.

(5) For sewage disposal, one of the bacterial methods, with application of the effluent to land, seems particularly applicable.

Experiments in India have already shown that even with a dilution as low as 3 gallons per head a satisfactory amount of purification can be obtained¹; the effluent being non-offensive and non-putrescible. While in England the criterion of the results of these methods is that the effluent shall be sufficiently pure, as measured by chemical standards, to allow it to be discharged into rivers, in India this will seldom be necessary. Irrigation is necessary during a considerable part of the year in almost all parts: this in fact is one of the chief obstacles to the profitable application of crude night-soil to the land. The same degree of purification need not, therefore, be insisted on. A non-putrescible effluent, in which the organic matter has been reduced to constituents which can be readily assimilated by growing plants is all that is required; and such an effluent has been shown to have considerably greater manurial value than crude sewage. In fact the water of the sewage, which is an obstacle to its disposal in England, would be an advantage in India.

To determine which of the various methods available is best suited to the requirements of Indian cantonments will require careful experiments. A closed septic tank, followed by continuous filtration, in connection with which I had the opportunity of making some experiments, gave good results, both as regards the condition of the effluent and the amount of labour required; and there are many points in favour of this method.

In combating the disease in armies in the field the principles remain the same, though the means that can be adopted must vary somewhat.

¹ James, C. C., "Notes on sewage disposal," 1901. *Ibid.* "Further notes on sewage disposal," 1901. Silk, A.E., "A sewage disposal experiment in Calcutta," 1900. Roberts, E., "On some practical methods of sanitation in India," 1901. By Director-General of Agriculture, Bombay, "Report on the cropping experiments at Manjri," 1901.

THE NATURE OF BERI-BERI.

AN ETIOLOGICAL STUDY AMONG CHINESE PRISONERS
IN SHANGHAI.

(One Figure.)

BY ARTHUR STANLEY, M.D., B.S. (LOND.), D.P.H.

Health Officer of Shanghai.

SHANGHAI is situate in the sub-tropical zone and has a climate of wide variation of temperature, but is broadly tropical during the summer quarter and temperate for the rest of the year. Lying on the alluvial flat of the Yangke delta the conditions of soil and sub-soil are uniform in all parts of the Settlement. The ground water-level is about five feet below the surface.

As a rule beri-beri becomes prevalent at the end of the summer, and is in Shanghai an autumn disease, though a few cases may occur throughout the year (see chart of seasonal prevalence).

The following observations extend from 1898 until the present time, and include 500 cases of beri-beri under my own care at the Shanghai Municipal Isolation Hospital. The cases were furnished by the Chinese prisoners under municipal supervision, incarcerated in the Gaol and in cells at three police-stations completely isolated from each other as regards situation and staff. Each of these places furnished numerous cases of beri-beri, the incidence being of approximately the same degree but greatest where the length of incarceration of the prisoners was most prolonged, namely, at the Gaol. The cases were furnished almost entirely by prisoners undergoing sentences of one month and over: of these 14% developed the disease, and broadly the tendency to acquire it was greater in proportion to the length of sentence. In many of the prisoners with sentences of two or more years the disease recurred each autumn. Each of the prisons is separated by a wall from a dense native population, but in this respect the isolation of one of them—the Gaol—is more complete than that of the others.

The conditions under which the prisoners live as regards ventilation, cleanliness, exercise, and food, are as a rule better than prior to incarceration. In the Gaol two prisoners sleep in a cell, and at the police-stations about a dozen sleep together in a much larger cell. Notwithstanding this propinquity whereby disease could easily be

CASES OF BERI-BERI ADMITTED INTO MUNICIPAL ISOLATION HOSPITAL.

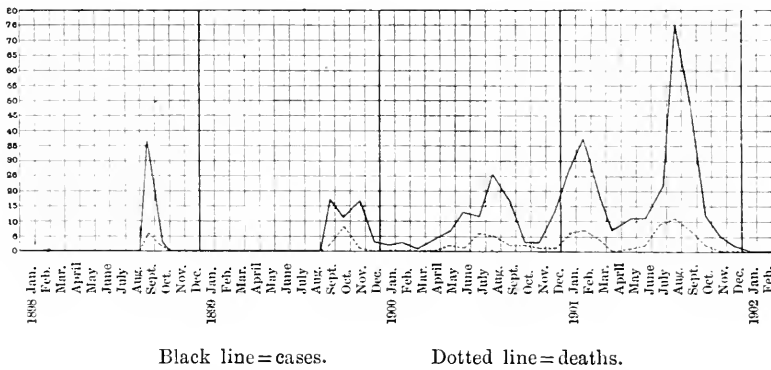


CHART SHOWING SEASONAL INCIDENCE WITH MAXIMUM AT END OF SUMMER.

propagated by contagion, beri-beri has been the only prevalent disease, and, with the exception of contagious impetigo due to lice, there was remarkably little disease other than beri-beri. Signs of beri-beri were rarely detected among the prisoners on admission, and the majority of cases developed the disease a month or more after incarceration.

GENERAL INCIDENCE OF BERI-BERI AMONG MUNICIPAL PRISONERS, 1898—1901

Total municipal prisoners	50,000
Prisoners with sentence of one month and over (these prisoners furnished all the cases of beri-beri)	3,430
Cases of beri-beri	480
Incidence of beri-beri on all prisoners	1%
Incidence of beri-beri on long sentence prisoners (<i>i.e.</i> with sentence of one month and upward)	14%
Deaths from beri-beri	98
Case-fatality	20%

DISTRIBUTION OF BERI-BERI AMONG PRISONERS

		Gaol	Station No. 1	Station No. 2	Station No. 3	Total
1898	Prisoners undergoing sentence of one month and over	Not yet opened	346	187	188	721
	Cases of beri-beri		15	19	6	40
	Deaths from beri-beri		2	6	2	10
1899	Prisoners undergoing sentence of one month and over	235	342	266	403	1,246
	Cases of beri-beri	27	3	5	13	48
	Deaths from beri-beri	7	1	2	1	11
1900	Prisoners undergoing sentence of one month and over	210	313	221	179	923
	Cases of beri-beri	34	53	55	7	149
	Deaths from beri-beri	12	12	10	0	34
1901	Prisoners undergoing sentence of one month and over	192	170	91	87	540
	Cases of beri-beri	134	60	30	19	243
	Deaths from beri-beri	18	11	13	1	43
Total prisoners furnishing cases		637	1,171	765	857	3,430
Total cases of beri-beri		195	131	109	45	480
Total deaths from beri-beri		37	26	31	4	98

PREVIOUS OCCUPATION OF PRISONERS SUFFERING FROM BERI-BERI

Coolies	158	Grocers	4	Ginseng seller	1
Boatmen	56	Coppersmiths	4	Merchant	1
Ricsha men	16	Masons	4	Tea-shop keeper	1
Carpenters	15	Fish sellers	4	Tin maker	1
Tailors	15	Sailors	3	Pawnshop broker	1
Soldiers	11	Wine sellers	3	News reporter	1
Barbers	10	Police constables	3	Farmer	1
Mafoos	10	Quartermasters	2	Hotel-man	1
Cooks	10	Tea merchants	2	Goldsmith	1
Blacksmiths	9	Teachers	2	Earthenware dealer	1
Painters	8	Tobacco dealers	2	Fish congee seller	1
'Boys'	8	Yamen runners	2	Gardener	1
Rice dealers	8	Chinese doctors	2	Filature coolie	1
Opium merchants	7	Meat sellers	2	Silk dealer	1
Professional thieves	7	Detectives	2	Scholar	1
Shroffs	5	Servants	2	Hot water seller	1
Shoemakers	5	Washermen	2	Lime maker	1
Detectives	5	Money-changers	2	Bookseller	1
Medicine sellers	4	Shopkeepers	2	Type picker	1
Brass smiths	4	Cotton-mill worker	1	Fan dealer	1
Bankers	4	Earthenware maker	1		

This table tends to show that beri-beri is not associated with any particular trade or occupation.

Comparative Prevalence in Gaols etc. outside Shanghai.

In the Singapore Gaols⁽¹⁾ in 1900 the incidence of beri-beri among a total number of 4,390 prisoners was 5.1%. In the Straits Lunatic Asylum half the total mortality was caused by beri-beri. In the Japanese Navy and in Japanese Gaols⁽²⁾ the percentage of cases of beri-beri varied from 10 to 30% previous to 1884. At the Hong Kong Gaol⁽³⁾ during 1900 out of a total number of 5,432 prisoners there were but 5 cases of beri-beri. Among the 110,016 prisoners in the whole of the Gaols of India⁽⁴⁾ during 1900 there were but 18 cases of beri-beri and 2 deaths. The maximum incidence of beri-beri is in Japan, but it is also very prevalent in the Malay Archipelago, and the Dutch East Indies. There is no beri-beri in the Gaols of England, though it has been claimed that an isolated outbreak has occurred at the Richmond Lunatic Asylum in Ireland.

Prevalence of Beri-beri in Shanghai, especially in other Institutions where Chinese are aggregated.

That the incidence of beri-beri among the Chinese public in Shanghai is not so great as among municipal prisoners is clear. Were the outside public to die from beri-beri at the same rate as the muni-

cipal prisoners do, there would be 17,500 deaths annually from this cause alone, while the total number of deaths for the year is under 5,000 from all causes. There is therefore an exceptional incidence of the disease among the municipal prisoners. The total number of deaths from beri-beri among the Chinese population in Shanghai from January to August 1901 given by the Chinese Death Register was 58, but little reliance can be placed on the accuracy of these figures. To determine the actual general prevalence of the disease the native institutions of Shanghai, where Chinese are aggregated, were visited.

Gaol under control of the Chinese authorities in the walled city of Shanghai. There were 70 prisoners here at the end of August, 9 of whom presented obvious signs of beri-beri. The prisoners lived under comparatively healthy conditions; each prisoner occupying one cell with windows of paper, which in the summer was allowed to wear away, so that day and night were spent in the open air. There was no deficiency of ventilation or light, and the prisoners looked comfortable. In fine weather they worked outside, making match-boxes. The food given in no way differed from that of an ordinary Chinese coolie, consisting of about $1\frac{1}{2}$ lbs. of rice daily, with cooked vegetables, fish, and seasonings.

Refuge under Chinese management for sick and aged. Here was an aggregation of some 300 starvelings, maniacs, lepers, and aged of both sexes. About 10% showed obvious signs of beri-beri. Ventilation and light were ample and the inmates were comfortably cared for.

Orphanage for Chinese, under control of Catholic Mission. This was visited in the autumn of 1900 and a large number of severe cases of beri-beri were found among the Chinese girls educated in it. The girls appeared to have every care and were provided with good food, but their bedrooms were overcrowded and badly ventilated.

Licensed Chinese prostitutes. No case of beri-beri was met with among some 250 licensed Chinese prostitutes who have been examined weekly for three years. These women may be taken as affording an instance of life among the general population, *i.e.* non-institutional life.

Medical men in practice locally among the Chinese rarely see cases of beri-beri among the better class of the general population. Cases however occasionally present themselves at the two native hospitals, but the prevalence is manifestly very much less among the Chinese public than among those aggregated in institutions.

Beri-beri therefore is chiefly found in Shanghai where the natives are aggregated together for long periods of time.

*The Nature of Beri-Beri**The Causation of Beri-beri.**General Considerations.*

The essential to prevention is a knowledge of the cause. With regard to beri-beri this knowledge is still conspicuously absent. Cantlie (1901) in the article on beri-beri in *Allchin's Medicine*, says: 'The cause of beri-beri has not yet been ascertained.' My own work up to the present time has been to some extent disappointing; the conclusions arrived at rather eliminating negative than establishing positive points. It is however of considerable value to discover with certainty what is not the cause, both for conserving energy in preventive measures, and as a means of being finally led into the way of truth. In spite of the fact that the cause of beri-beri may be considered to be as yet unknown, the question may still be asked—What determines the exceptional incidence of the disease among municipal prisoners in Shanghai? Why should beri-beri be more common in native institutions than among the general population?

Possible Infection in Beri-beri.

If the disease were communicable it would have every opportunity for spreading among the prisoners in the Gaol and police cells. But practically all observers of beri-beri are at one in considering infection a negligible danger. There can be no doubt, however, that beri-beri is apt to be an institutional disease, which favours the conclusion that infection may after all play a part in its spread. In this respect it may be regarded as analogous to diphtheria, which may be limited to individual houses; but when there is aggregation of susceptible units, as in a school, the disease may spread rapidly. The infection here only becomes obvious where there is aggregation of susceptible units.

The Gaol and Police-stations are inhabited by a series of individuals any one of whom may introduce the cause. The criminal class is more likely to be infected than any other. There is therefore a greater probability of these places becoming infected than any ordinary dwelling-house inhabited by one family, which is a unit more or less isolated. This applies to all similar institutions where there is an aggregation of changing units. The length of sojourn of the prisoners, especially the longer term prisoners of the Gaol, is also in favour of development of a disease like beri-beri, which has a prolonged incubation period.

The evidence usually adduced of the absence of infection in beri-beri is afforded chiefly by the fact that hospital assistants do not contract the disease from the patients. The infection may however require intimate and prolonged contact or the infection may be conveyed by parasites—bugs, fleas and lice—which are most common among the criminal class.

The Isolation Hospital furnishes strong evidence against mosquitoes propagating the disease. No case of beri-beri has arisen among the sick prostitutes, who are kept in wards contiguous to the beri-beri wards. Moreover mosquitoes, especially *Culex fatigans* and also *Anopheles sinensis*, are very numerous at the Isolation Hospital.

With regard to lice it is the rule to find the pigmentation of the skin and pustular impetigo, which is associated with body lice—so called ‘Vagrant’s disease.’ Here therefore is an adequate means of conveying infection from one to another, especially when more than one prisoner sleeps in the same cell.

Beri-beri and Locality.

The Gaol and the three Police-stations, more or less widely separated from each other, were nearly equally affected with beri-beri. The fact that these were nearly equally affected by the disease practically eliminates the idea of miasma of limited localisation. Moreover at the Police-stations numbers of European and Indian police reside, among whom no case of beri-beri has arisen. The cause of the disease therefore does not directly arise either from the site or its immediate surroundings. It does not therefore arise directly from the soil.

Beri-beri and Food.

Rice was found to be the only food common to all the Police-stations. The Gaol source was however a different one. The remaining articles of food—fresh and salt vegetables, fish, pork and oil—were obtained from different sources.

Rice was the suspected article of food inasmuch as other cereals, e.g. rye and maize, when infected by a fungus, may cause symptoms of poisoning analogous to beri-beri,—ergotism or pellagra. The rice used at the various stations was examined and found to be of very poor quality, weevilily, and with a distinct mouldy smell.

In order to ascertain whether this inferior rice was a cause of the

disease it was recommended that a common supply of rice of a recognised good quality be obtained. With this object an excellent quality of rice was obtained from Annam—long-grained rice—at \$5³⁰ a picul. The mouldy rice then in use cost \$4⁶⁰ a picul. 100 piculs of this Annam rice was purchased and stored at the Central Station in a concrete cell, which is sometimes used for foreign prisoners. All openings of this cell were closed and the atmosphere was kept dry and pure by means of numerous open jars of chloride of lime, round which the sacks of rice were piled. This supply of rice was distributed every three days to the Gaol and Police-stations from the beginning of August until well into November. In view of the long incubation period however no diminution of cases was expected or occurred in August. Toward the end of September however the cases diminished markedly in number, and in October the disease almost ceased. But this may also be explained by seasonal diminution, beri-beri in Shanghai being essentially a disease of late summer and early autumn. (See chart of seasonal incidence.) The results of the rice experiment were therefore probably negative. Annam rice was used rather because its long grains were easy to identify and one could make certain that it was actually being used. Annam is by no means free from beri-beri. In fact, in practically all rice-producing countries beri-beri is prevalent, so that the possibility of rice carrying the infection can with difficulty be eliminated. The conditions of the experiment therefore preclude any definite conclusions.

The history of beri-beri in the Japanese Navy, Army, and Prisons, is interesting, inasmuch as in Japan it is firmly believed that the nature of the diet rather than any definite infective agent is the cause of beri-beri. The Japanese claim to have reduced the prevalence of beri-beri from 20% to 1% by a change of diet from almost exclusively rice to one containing rice in a comparatively small quantity; the deficit being made up by wheat, barley, beans, and meat, *i.e.*, by a diet of more albuminous and fatty character⁽²⁾.

In the prisons of Japan the daily allowance of rice was 750 grammes with some poor auxiliary food, costing 1 to 1.5 sen per head per day. In 1875 the prisoners were allowed barley also, and in 1881 the proportion of barley to rice was as 6:4. Since this change was made beri-beri is said to have become rare. The experiment was repeated in the Japanese Army, with the result that the number of cases occurring there was reduced to 1%.

On account of a short crop in Japan in 1899 rice imported from China was used for the prisoners of Nagata, and from January to

March 400 out of 1000 prisoners had beri-beri. It is said that those supplied with 'white Chinese rice' were more affected than those supplied with 'red Chinese rice.'

In Corea, where the Japanese and Coreans live side by side, the Japanese commonly have beri-beri, while it occurs only very rarely among Coreans. The Corean houses are small, dark and dirty, but the inhabitants feed chiefly on peas and do not eat rice as the Japanese do. Beri-beri is common in Brazil, where rice is the staple food.

It is held by the Japanese that beri-beri always prevails where rice is the principal food of the nation. Beri-beri however is very much less prevalent in India than in Japan.

In the Straits Settlements the Tamils are very slightly affected by beri-beri, while the incidence among the Chinese and Malays is marked. Rice is the staple food of each, but the Tamil decorticates his rice after cooking, while the Chinese and Malays eat rice which has been husked up to a year or more previous to use⁽⁸⁾.

The theory that beri-beri is due to the use of food, insufficient or unsuitable, but not specifically contaminated does not explain the seasonal variation of the disease. Nor is it likely that beri-beri, which is essentially a peripheral neuritis, a pathological condition usually associated with toxæmia, would be caused merely by insufficiency of food were this in itself wholesome. Should however the rice be specifically contaminated it is conceivable that the replacement by uncontaminated food, whether of another variety or not, would stop the disease.

The Japanese explain the excessive prevalence in summer by the appetite desiring the plainer foods, such as rice, to the exclusion of more albuminous and fatty foods. The excessive prevalence in barracks, gaols and other crowded places they assign to the food being given regardless of the choice of the individual with a consequent increase in the quantity of rice. The Japanese view regarding the causation of beri-beri is not generally accepted. Still it is quite probable that a diminution in the rice consumed may actually be the cause of a diminution of beri-beri: the reason being that rice, although unable of itself to produce the disease may yet carry its specific contagium. Beri-beri is quite prominently a Japanese disease. This being so it has been thought proper to adopt the Japanese method of stopping it, and it is at the present time being applied at the Municipal Gaol. About half the ration of rice is replaced by beans and crushed wheat or barley. Inasmuch as the change was instituted when cases of beri-beri naturally decline in number, another year must elapse before any conclusion can

be drawn. It may be said however that since the change of diet was made no fresh cases of beri-beri have arisen.

BERI-BERI IN THE JAPANESE NAVY.

Year	Cases of beri-beri per 100 of Navy			Grammes
1878	32.8	Food ration during these years was	Rice	782
1879	38.9		Fish	96
1880	34.8		Beef	73
1881	25.0		Pickled vegetables	145
1882	40.4		Fresh vegetables	215
1883	23.1		Sugar	18
1884	12.74		Bean sauce	60

The ration was here changed for a more albuminous and fatty one.

1885	0.59	Food ration during these years was	Rice (or 600 bread or 490 biscuits)	648
1886	0.04		Fish	150
1887	0.0		Fresh vegetables	450
1888	0.0		Milk	45
1889	0.03		Sugar	75
1890	0.04		Meat	300
1891	0.01		Bean sauce	50
1892	0.03		Flour (wheat)	75
1893	0.01		Beans	15
1894	0.26		Pickled vegetables	75
1895	0.13		Fat	15
1896	0.08		Salt	8
1897	0.14		Tea and vinegar	
1898	0.08		Saké	90

Experimental Work.

Bacteriological Examination of the Blood in Beri-beri.—Thirty cases, where the symptoms were well-marked and in stages both before and after loss of knee-jerks, were examined. A band being placed round the arm to distend the veins, the bend of the elbow was sterilised by 10% lysol in strong alcohol repeatedly rubbed in for half-an-hour and then washed with ether. The needle of a sterile all-metal syringe was plunged into the median cephalic vein and 1 c.c. of blood withdrawn. This blood was examined under the microscope directly and also stained with methylene blue with a negative result. Tubes of peptone bouillon, gelatin, agar and blood serum were inoculated with two or three drops of blood in each: deep stabs in glucose-agar were also made. Beyond the adventitious inoculation by *Staphylococcus aureus* and *M. tetragenus* respectively, of two out of 150 tubes incubated all remained sterile.

Six rabbits were injected subcutaneously with 1 c.c. of blood from six well-marked cases of beri-beri but nothing resulted.

Diet experiments.—We have seen (p. 376) that the exclusive use of

Annam rice for three months by all the prisoners caused no reduction of cases other than would be explained by seasonal variation.

Storage of rice.—The grain in the husk keeps for two or three years with comparatively little care, but when decorticated greater delicacy is necessary to preserve the grain intact. Granaries for decorticated rice are usually raised well above ground, the rice being stored in large hollow baskets so as to avoid damp, and sometimes the ash of the burnt husk is used to scatter among the grains as an antiseptic. Before being sold in the market the rice undergoes, besides decortication, a pounding with wooden hammers, which makes it white by bruising the outer coat of the grain, fracturing also the pointed end: by this process the outside of the grain becomes coated with floury matter, and it is this which is washed away during the process of 'washing the rice,' which is invariably done immediately before cooking by placing the rice in a fine wicker basket and immersing and rinsing it several times in water. There is apparently no definite reason why this should be done, for good starch in large quantities is thus wasted; and this among a wonderfully thrifty people like the Chinese is remarkable. It is interesting to trace the evolution of these methods of treating rice. The processes of preserving and treating the rice grain, which in China have been arrived at through a period of domestic evolution of some thousands of years, point to the use of methods now recognised to be antiseptic and having for their object the preservation of the grain from vegetable parasites, *e.g.* retention of the husk as long as possible, dry storage, use of ash as an antiseptic, detrition of the outer layer of the decorticated grain and subsequent washing away of the detritus, and finally sterilising by prolonged boiling during cooking. In this way the rice is preserved from parasites and their products, and before being consumed it is thoroughly sterilised. It is therefore possible only to introduce with the rice into the human body the products of an organism which may have established itself in the grain, much as ergot of rye or maize infected with pellagra or beans with lupinosis.

Rice used by Chinese prisoners furnishing the present series of cases.—The rice was found to be old rice of a cheap quality with a distinct mouldy smell. There were the usual proportions present of red and blue grains found in most local samples of rice. Rough bacteriological examination yielded nothing distinctive as to flora.

Five mice were fed with the unboiled rice for a period of two months without effect.

Six guinea-pigs were fed with the boiled rice for two months without result.

An aqueous extract, made by boiling and concentrating to small bulk, was injected weekly for three weeks into four rabbits with no ultimate result.

Alcoholic and glycerin extracts were also made and injected into rabbits without anything noteworthy happening.

CONCLUSIONS.

1. The incidence of beri-beri in Shanghai on Chinese prisoners under municipal police supervision is markedly greater than on the general public.

2. The incidence in four widely separated prisons completely isolated in every respect was of approximately the same degree. In none of these places were the European and Indian staff affected though they resided in the same compound with the prisoners. The cause of the disease therefore does not arise either from the soil or its immediate surroundings.

3. The simultaneous incidence at the Gaol and Police-stations would point rather to a general cause than to place infection; but would also be explained by diffuse infection among the native community generally; a case admitted to aggregations of susceptible units as in Gaol and police cells spreading by contagion (intimate contact).

4. The figures show a progressive development of infectivity of beri-beri on all the four places where municipal prisoners are aggregated.

5. The fact that beri-beri mainly occurs among natives aggregated for periods of over one month favours the idea of its propagation by contagion. Given the presence of the infective agent, whether conveyed in food or by parasites or by contagion, its operation would be favoured by aggregation of potentially infective units.

6. Inasmuch as, apart from rice, the food supply of three out of the four prisons was from different sources and a change of rice for all the prisons to one of recognised good quality produced no well-marked effect on the prevalence of the disease in two months, food infection would appear not to be a factor in the cause.

7. Beri-beri being a peripheral neuritis, which is a pathological condition usually associated with toxæmia, food would in the absence of a primary lesion (as in diphtheria) seem specially indicated as a cause. For the same reason the cause would be met with in specifically contaminated food rather than in either qualitative or quantitative changes in diet.

8. The marked and apparently primary degenerative action of beri-

beri on heart muscle like that produced by diphtheria⁽⁵⁾ and to a less degree by influenza and alcohol and arsenic poisoning, all of which may also cause peripheral neuritis, and the remarkable clinical resemblance of beri-beri to diphtheria in the frequency of death from sudden heart failure would indicate a form of chronic poisoning⁽⁶⁾.

9. The identity of the pathological changes in beri-beri, diphtheria, and arsenic and alcohol poisoning and the grouping of alcoholic poisoning with ergotism, pellagra, and lathyrism, which are caused by poisons produced by parasites in vegetable foods, suggest the possibility of the cause of beri-beri being a toxine derived from an extraneous parasite of some article of food.

10. In the outbreak in Richmond Asylum, Ireland, which is held to disprove the rice origin of the disease, it is impossible to eliminate from the diet such articles as rice, tapioca, sago, etc. which may have been derived from countries in which beri-beri is prevalent. Beri-beri appears to be markedly prevalent only in countries where rice is the staple food.

11. The oft-repeated statement that a beri-beri patient recovers quickly when removed to a fresh locality may not indicate that this disease is a place infection, but rather that the source of the toxine may be removed by change of residence.

12. Beri-beri does not appear to be associated with any particular trade or occupation.

13. Something more is required for the prevention of beri-beri than attention to the general rules of sanitation, such as ventilation, cleanliness and diet. Moreover isolation of cases as they arise, followed by disinfection, does not suffice to limit the disease.

14. The maximum incidence of beri-beri in Shanghai being at the end of the tropical summer (the remainder of the year being quite temperate) the liability to recurrence yearly at this season in the same patient would be compatible with the elaboration of a toxine favoured in its origin by the period of maximum atmospheric heat and moisture.

15. The blood in beri-beri is sterile.

16. The bacteria found by Pekelharing and Winkler⁽⁷⁾ associated with beri-beri bear no causal relation to the disease.

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IN MEMORIAM.

PATRICK THURBURN MANSON.

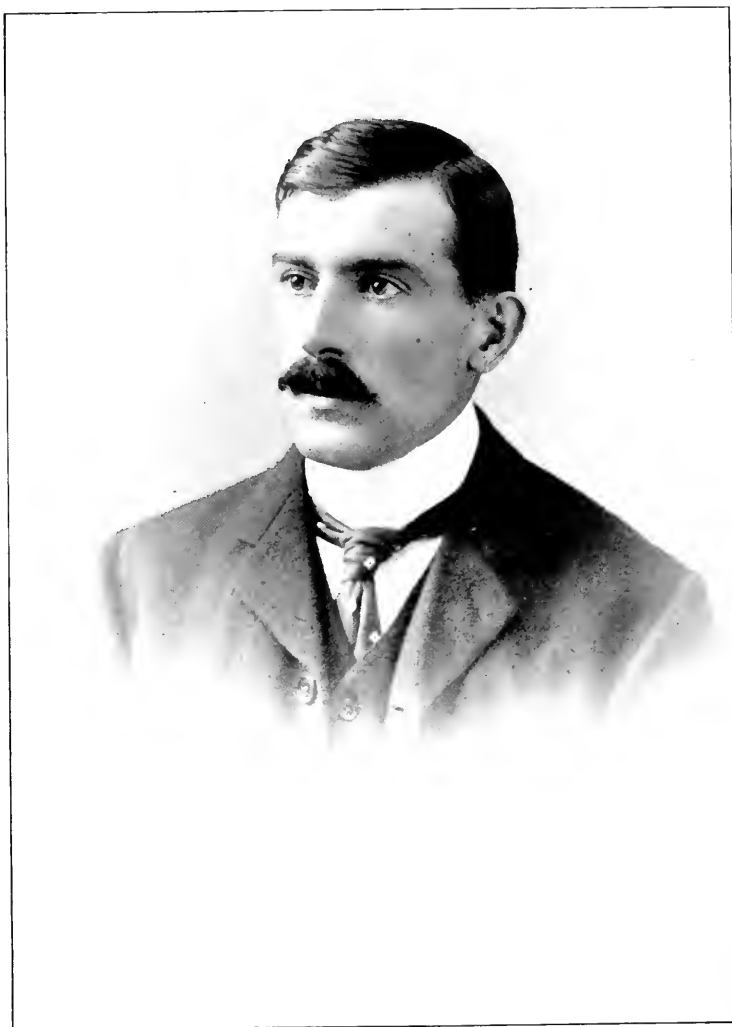
THE sudden death of Patrick Thurburn Manson has left a gap in the ranks of the promising young investigators of this country. The widespread regret felt by those who knew him or his work bears fitting testimony to the esteem in which he was held both for himself and as his father's son.

He was born on the 20th of August, 1878, in Amoy, China, where his father had recently made his great discovery of the development of *Filaria bancrofti* in the mosquito. Young Manson was educated at Harrow, and entered Guy's Hospital in 1895. In 1900 he took the degree of M.B. at London University, and subsequently studied pathology under Professor Hamilton of Aberdeen, after which he went to the London School of Tropical Medicine.

In 1900 he submitted himself to the crucial experiment through which he will be remembered in the history of medicine¹. He exposed himself to the bites of infected *Anopheles* sent from Rome by Bignami and Bastianelli. The insects had previously sucked the blood of a patient suffering from mild tertian malaria. The result of the experiment was that Manson developed tertian fever, the parasites being found in his blood. The initial infection was followed by two recurrences, the first at Aberdeen during the summer of 1901, the second whilst he was out on a holiday shooting. The attacks were cut short through the administration of quinine. This experiment removed the doubts of those sceptics who remained unconvinced by the similar infection experiments of Grassi, Bignami and Bastianelli, on the ground that the Italian experiments were conducted in a country where malaria was indigenous.

¹ Manson, P. (29 Sept. 1900), Experimental Demonstration of the Mosquito-Malaria Theory. *Brit. Med. Journ.*, vol. ii., pp. 949—951; *Lancet*, vol. ii., pp. 923—925.

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PATRICK THURBURN MANSON

Born at Amoy, China, 20 August, 1878.
Died at Christmas Island, Straits Settlements,
8 March, 1902.

The esteem in which he was held by his fellow-students is clear from what one of them wrote of him¹: "Mature beyond his years in mind and physique, he always impressed those who worked with him with his whole-heartedness, his clearness of thought, his distaste for inaccuracy and bluff. Full of energy and resource, steadfast in purpose, he was as enthusiastic in the field of work as in that of sport; while there was such a bright vein of gaiety in his nature that life was to him a source of perpetual enjoyment, and with this joy of living he infected all his surroundings—all his associates." One who knew him best tells us, "He was a good student, but not a prize-taking student; remarkable for sturdy common sense and grasp rather than for brilliancy. He was a first class clinical man and very conscientious in diagnosis and in carrying out what he conceived to be his duty to his patient."

In January, 1902, young Manson left England to join Dr Herbert E. Durham at Christmas Island, Straits Settlements, their common object being to study beri-beri. Soon after his arrival at his destination he met with a gun accident which resulted fatally on the 8th of March. He was buried in Hong-Kong.

The general regret caused by the news of his death is mingled with feelings of the deepest sympathy for his family, more especially for his distinguished father, who would have had a worthy successor in the son whose career of promise has been cut off all too soon.

G. H. F. N.

¹ Obituary Notice in *Guy's Hospital Gazette*, vol. xvi., p. 139.

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¹ The size of books is given roundly in centimeters. Ed.

ON THE BACTERICIDAL EFFECT EXERTED BY HUMAN
BLOOD ON CERTAIN SPECIES OF PATHOGENIC MICRO-
ORGANISMS AND ON THE ANTIBACTERICIDAL EFFECTS
OBTAINED BY THE ADDITION TO THE BLOOD IN
VITRO OF DEAD CULTURES OF MICRO-ORGANISMS IN
QUESTION.

By A. E. WRIGHT, M.D.,

Professor of Pathology, Army Medical School, Netley;

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THE fact that the blood of ordinary laboratory animals exerts a very marked bactericidal effect upon the *Bacillus typhosus* and the *Spirillum cholerae asiaticae*, while it exerts little or no effect upon the *Staphylococcus* and *Streptococcus pyogenes*, has hardly received the attention which it would seem to merit in view of the circumstance that these facts involve the important problem as to whether the blood exerts its bactericidal action upon pathogenic organisms generally, or only upon certain species of such micro-organisms.

We have addressed ourselves to the task of re-investigating this general problem by the aid of the methods of bactericidal estimation which have been elsewhere described by one of us¹, conducting our experiments upon human blood, and drawing within the sphere of our observation, not only the micro-organisms particularized above, but also the *Micrococcus melitensis* of Bruce and the *Bacillus pestis*.

I. *Data with regard to the bactericidal power of the blood as affecting the Bacillus typhosus and Spirillum cholerae asiaticae.*

We may begin by setting forth certain data in connection with the bactericidal power of human blood upon the *Bacillus typhosus*, and

¹ *Lancet*, June 1, 1901, p. 1532; *Proc. Roy. Soc.* (this paper is about to appear).

Bactericidal Effects of Blood

TABLE I.
Exhibiting (a) the bactericidal action exerted by human serum on Bacillus typhosus and (b) the antibactericidal effect obtained by the addition of a sterilized typhoid culture to the mixture of serum and living culture.

Capillary testing pipettes were filled with mixture of												
Dilutions in which the living typhoid culture was employed	2 vols. F. N. W.'s serum 1 vol. dilution of living culture which contained 33,000,000 <i>T.B.</i> per c.c. and		1 vol. H. B.'s serum 1 vol. dilution of living culture which contained 220,000,000 <i>T.B.</i> per c.c. and		2 vols. A. E. W.'s serum 1 vol. dilution of living culture which contained 210,000,000 <i>T.B.</i> per c.c. and		1 vol. H. B.'s serum 1 vol. dilution of living culture which contained 220,000,000 <i>T.B.</i> per c.c. and		1 vol. A. E. W.'s serum 1 vol. dilution of living culture which contained 260,000,000 <i>T.B.</i> per c.c. and		1 vol. A. E. W.'s serum 1 vol. dilution of living culture which contained 340,000,000 <i>T.B.</i> per c.c. and	
	1 vol. sterile broth 1 vol. sterilized typhoid culture	1 vol. sterile broth 1 vol. sterilized typhoid culture	1 vol. sterile broth 1 vol. sterilized typhoid culture	1 vol. sterile broth 1 vol. sterilized typhoid culture	1 vol. sterile broth 1 vol. sterilized typhoid culture	1 vol. sterile broth 1 vol. sterilized typhoid culture	1 vol. sterile broth 1 vol. sterilized typhoid culture	1 vol. sterile broth 1 vol. sterilized typhoid culture	1 vol. sterile broth 1 vol. sterilized typhoid culture	1 vol. sterile broth 1 vol. sterilized typhoid culture	1 vol. sterile broth 1 vol. sterilized typhoid culture	
undiluted	—	—	—	—	—	—	—	—	—	—	—	
2-fold dilut.	growth	growth	growth	growth	growth	growth	growth	growth	growth	growth	growth	
5 "	"	"	"	"	"	"	"	"	"	"	"	
10 "	sterile	sterile	sterile	sterile	sterile	sterile	sterile	sterile	sterile	sterile	sterile	
25 "	"	"	"	"	"	"	"	"	"	"	"	
50 "	"	"	"	"	"	"	"	"	"	"	"	
100 "	"	"	"	"	"	"	"	"	"	"	"	
1000 "	sterile	sterile	sterile	sterile	sterile	sterile	sterile	sterile	sterile	sterile	sterile	
10,000 "	"	"	"	"	"	"	"	"	"	"	"	
100,000 "	"	"	"	"	"	"	"	"	"	"	"	

F. N. W. and H. B. were normal men. A. E. W. had 9 months previously, and also on previous occasions, undergone anti-typhoid inoculation. In every case, both in this and in the subsequent tables, the serum was employed within 2 or 3 hours after the blood had been drawn off. In every case, both in this and in the subsequent tables, the living cultures employed were, unless otherwise stated, 24 hour old broth cultures. Here and in the subsequent tables the sterilized bacterial cultures employed had been sterilized by exposure to a temperature of about 60°–65° C. for 10–20 minutes.

Here and in the subsequent tables the sera were allowed to act upon the bacteria with which they were mixed for 18–24 hours at 37° C. before the effect was tested.

In every case the term "growth" denotes that the growth obtained presented the characters of a pure cultivation of the micro-organisms employed in the test. In cases of doubt the purity of the culture was tested by subcultures and microscopical examination. Where contaminations were found the series of experiments was in almost every instance rejected. Where such a series is retained the fact that a contamination was found is in each case specifically mentioned.

TABLE II.

Exhibiting the antibactericidal effects produced by the addition to a mixture of serum and living typhoid culture of filtrates from typhoid cultures.

Dilutions in which the living typhoid culture was employed	Capillary testing pipettes were filled in with					
	1 vol. F. N. W.'s serum of living culture which contained 75,000,000 T.B. per c.c. and			1 vol. A. E. W.'s serum of living culture which contained 75,000,000 T.B. per c.c. and		
	1 vol. dilution of living culture	1 vol. sterile broth	1 vol. same 5 months old typhoid culture unfiltered	1 vol. dilution of living culture	1 vol. sterile broth	1 vol. filtrate from a 5 months old typhoid culture
	1 vol. sterile broth	1 vol. filtrate from a 24 hours typhoid culture	1 vol. filtrate from a 5 months old typhoid culture	1 vol. filtrate from a 24 hours typhoid culture	1 vol. filtrate from a 24 hours typhoid culture	1 vol. filtrate from a 5 months old typhoid culture
2-fold dilut.						
5 "	growth	growth	growth	growth	growth	growth
10 "	sterile *	"	"	"	"	"
25 "	growth	"	"	"	sterile	sterile
50 "	sterile	sterile	"	"	"	"
100 "	"	"	"	"	"	"
1000 "	"	"	sterile	sterile	"	"
10,000 "	"	"	"	"	"	contamination +
100,000 "	"	"	"	"	"	sterile
1,000,000 "	"	"	"	"	"	"

With regard to F. N. W. and A. E. W. and general condition of the experiments see notes to Table I.

* Sterility of the tube probably due to accidental overheating.

+ *Staphylococcus*.

with regard to the "antibactericidal effect" obtained by the introduction of a sterilized culture of the typhoid bacillus into human blood *in vitro*¹.

A point of incidental interest here suggests itself in connection with the question as to what is the element in the sterilized culture which exerts the antibactericidal effect exemplified in Table I.

The experiments subjoined in Table II. are typical examples of a number of experiments instituted with a view to the determination of this question.

These results show that a filtrate from a young culture of *B. typhosus* exerts little or no antibactericidal effect; while a filtrate from an old culture which contains in solution elements derived from the dissolution of the typhoid bacilli exerts a very marked antibactericidal effect. Of particular interest are the results in columns 3 and 4, which show that the filtrate derived from a culture in which the bacilli had been macerating at 37° C. for a period of 5 months, diminished the bactericidal power of the serum with which it was mixed to exactly the same degree as the unfiltered culture.

Passing to the consideration of the bactericidal effect exerted by human serum upon the cholera vibrio, we subjoin a selection of typical experiments illustrating on the one hand the bactericidal effect exerted upon the cholera vibrio, and on the other hand, the diminution of bactericidal power which is achieved by the addition of a sterilized cholera culture to a mixture of serum and living cholera culture.

It will be manifest from a comparison of the experiments in Table I. and Table III. that the bactericidal and antibactericidal effects proceed on precisely the same lines whether we are employing a culture of typhoid or a culture of cholera.

It becomes, therefore, a point of interest to determine whether a diminution of the bactericidal effect exerted on the typhoid bacillus is obtained by the addition of a sterilized cholera culture to the mixture of serum and living typhoid culture; and *vice versa* whether a diminution of the bactericidal effect exerted on the cholera vibrio is

¹ Data with regard to the first of these points have already been set forth by one of us in a paper published in the *Lancet*, Sept. 14th, 1901, p. 715, dealing with the changes produced in the blood by antityphoid inoculation. The second of these questions has also been briefly adverted to in the same Journal, June 1st, 1901, p. 1534, in connection with a suggestion that the antibactericidal effect exerted might serve as a criterion for the standardization of bacterial vaccines.

TABLE III.

Exhibiting (a) the bactericidal effect exerted by serum on a 24 hour old culture of the cholera vibrio, and (b) the antibactericidal effect achieved by the addition of a sterilized cholera culture to the mixture of serum and living cholera culture.

	Capillary tubes were filled with					
	1 vol. F.N.W.'s serum 1 vol. dilution of living culture which contained 45,000,000 cholera vibrios per c.c. and	1 vol. A.E.W.'s serum 1 vol. dilution of living culture which contained 45,000,000 cholera vibrios per c.c. and	1 vol. serum of Rabbit no. 1 1 vol. dilution of living culture which contained 45,000,000 cholera vibrios per c.c. and	1 vol. serum of Rabbit no. 2 1 vol. dilution of living culture which contained 45,000,000 cholera vibrios per c.c. and		
Dilutions in which the living cholera culture was employed	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile- ized cholera culture
	growth	growth	growth	growth	growth	growth
undiluted culture	growth	growth	growth	growth	growth	growth
2-fold dilution	"	"	"	"	"	"
5 "	"	"	"	"	"	"
10 "	sterile	"	"	"	"	"
25 "	"	"	sterile	"	"	"
50 "	"	"	"	"	"	"
100 "	"	"	"	"	"	"
1000 "	"	"	"	"	"	"
10,000 "	"	sterile	"	"	"	sterile
100,000 "	"	"	"	"	"	"

With regard to F.N.W. and A.E.W. see notes to Table I.

Rabbit 1 had been inoculated with one tube of typhoid bacillus.

Rabbit 2 " " " " cholera vibrio.

TABLE IV.

Exhibiting (a) the bactericidal effect exerted on a typhoid culture and (b) the diminution of that effect which is achieved by the addition of a sterilized cholera culture.

Dilutions in which the living typhoid culture was employed	Capillary tubes were filled with							
	1 vol. F.N.W.'s serum 1 vol. dilution of 24 hr. old living broth culture of the typhoid bacillus and		1 vol. A.E.W.'s serum 1 vol. dilution of 24 hr. old living broth culture of the typhoid bacillus and		1 vol. serum of Rabbit 1 1 vol. dilution of 24 hr. old living broth culture of the typhoid bacillus and		1 vol. serum of Rabbit 2 1 vol. dilution of 24 hr. old living broth culture of the typhoid bacillus and	
	1 vol. sterile broth	1 vol. steri- lized cholera culture	1 vol. sterile broth	1 vol. steri- lized cholera culture	1 vol. sterile broth	1 vol. steri- lized cholera culture	1 vol. sterile broth	1 vol. steri- lized cholera culture
undiluted culture	growth	growth	growth	growth	growth	growth	growth	growth
5	"	"	"	"	"	"	"	"
10	sterile	"	"	"	sterile	"	"	"
25	"	"	sterile	"	"	"	"	"
50	"	"	"	"	"	"	"	"
100	"	"	"	"	"	"	"	"
1000	"	"	"	"	"	"	"	"
10,000	"	sterile	"	sterile	"	"	sterile	"
100,000	"	—	"	"	"	"	"	"
1,000,000	"	—	"	"	"	"	"	"

With regard to F.N.W., A.E.W., and Rabbits 1 and 2, see notes to Table III.

TABLE V.

Exhibiting (a) the bactericidal effect exerted on a cholera culture, and (b) the diminution of that effect produced by the addition of a sterilized typhoid culture.

Capillary testing pipettes were filled with					
Dilutions of the living cholera culture which were employed	1 vol. A. E. W.'s serum 1 vol. dilution of living cholera culture which contained 465,000,000 chol. vibrios per c.c. and	1 vol. serum of Rabbit 1 1 vol. dilution of living cholera culture which contained 85,000,000 chol. vibrios per c.c. and	1 vol. serum of A. E. W. 1 vol. dilution of living cholera culture and	1 vol. W. G. L.'s serum 1 vol. dilution of living cholera culture containing 2,000,000 chol. vibrios per c.c. and	1 vol. steri- lized typhoid culture
	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	
undiluted culture	growth	growth	sterile	growth	growth
2-fold dilution	"	"	"	"	"
5 "	"	"	"	"	"
10 "	"	"	"	"	"
25 "	sterile	sterile	"	"	"
50 "	"	"	"	"	"
100 "	"	"	"	"	"
1000 "	"	"	"	"	"
10,000 "	"	"	"	sterile	sterile
100,000 "	"	"	"	"	"

With regard to A. E. W. and Rabbit no. 1, see notes to Table III.

TABLE VI.

Exhibiting the results of the blood examinations carried out on two rabbits which were inoculated with similar quantities, in the one case of a sterilized typhoid culture, in the other case of a sterilized cholera culture. These rabbits are in the table below denoted respectively as the typhoid rabbit and the cholera rabbit.

	Tests carried out immediately antecedent to inoculation				Tests carried out 24 hours after inoculation			
	Capillary testing pipettes were filled in with				Capillary testing pipettes were filled in with			
	1 vol. dilution of a culture of typhoid which contained 34,000,000 T.B. per c.c.	1 vol. serum of typhoid rabbit	1 vol. dilution of a very thin culture of cholera	1 vol. serum of cholera rabbit	1 vol. dilution of a culture of typhoid which contained 446,000,000 T.B. per c.c.	1 vol. serum of typhoid rabbit	1 vol. dilution of a very thin culture of cholera	1 vol. serum of cholera rabbit
Dilutions of the living cultures which were employed in the testings	growth	growth	growth	growth	growth	growth	growth	growth
	"	"	"	"	"	"	"	"
undiluted culture	sterile	sterile	sterile	sterile	sterile	sterile	sterile	sterile
	5	"	"	"	"	"	"	"
	10	"	"	"	"	"	"	"
	25	"	"	"	"	"	"	"
	50	"	"	"	"	"	"	"
	100	"	"	"	"	"	"	"
	1000	"	"	"	"	"	"	"
	10,000	"	"	"	"	"	"	"
	100,000	"	"	"	"	"	"	"
	"	"	"	"	"	"	"	"

TABLE VI. (*continued*).

	Tests carried out 48 hours after inoculation				Tests carried out 14 days after inoculation			
	Capillary testing pipettes were filled in with				Capillary testing pipettes were filled in with			
	1 vol. dilution of a culture of typhoid which contained 324,000,000 T./b. per c.c. and	1 vol. serum of cholera rabbit	1 vol. serum of typhoid rabbit	1 vol. dilution of a culture of cholera which contained 400,000 C.F. per c.c. and	1 vol. serum of typhoid rabbit	1 vol. serum of cholera rabbit	1 vol. dilution of a culture of typhoid which contained 480,000,000 T./b. per c.c. and	1 vol. serum of cholera rabbit and 1 vol. serum of typhoid rabbit
Dilutions of the living cultures which were employed in the testings								
undiluted culture	growth	growth	growth	growth	growth	growth	growth	growth
2-fold dilution	"	"	"	"	"	"	"	"
5 "	sterile	"	"	sterile	"	"	"	sterile
10 "	"	"	"	"	"	"	"	"
25 "	"	"	"	"	"	"	sterile	"
50 "	"	"	"	"	"	"	"	"
100 "	"	"	"	"	"	"	"	"
1000 "	"	"	"	"	"	sterile	"	"
10,000 "	"	sterile	"	"	"	"	"	"
100,000 "	"	"	"	"	"	"	"	"

obtained by the addition of a sterilized typhoid culture to a mixture of serum and living cholera vibrios.

Tables IV. and V., which show the effect invariably obtained in our experiments, supply the answer to this question.

As shown in the Tables IV. and V., taken in conjunction with Tables I. and III., the antibactericidal effect which is in each case obtained, is obtained indifferently with either variety of sterilized culture. We must consequently assume either that the bactericidal substance in the serum which kills the typhoid bacillus is one and the same substance which kills the cholera vibrio, or alternatively, that the bactericidal substance which kills the cholera vibrio possesses an element in common with the bactericidal substance which kills the typhoid bacillus.

With a view to deciding between these alternatives, we have investigated the question as to whether the inoculation of a full dose of antityphoid vaccine, which produces in man a preliminary diminution and subsequent increase in the bactericidal effect exerted on the typhoid bacillus¹, brings about any similar diminution and increase in the bactericidal effect exerted upon the cholera vibrio.

The following observations bear on the question.

The bloods of three healthy men, who recently came up for prophylactic inoculation with antityphoid vaccine, were tested before inoculation and afterwards, at intervals of a few days, against both the typhoid bacillus and the cholera vibrio. In no case was any indication obtained of an alteration in the bactericidal effect exerted on the cholera vibrio, although the negative and positive phases of diminished and exalted bactericidal power with respect to the typhoid bacillus manifested themselves in a typical manner.

These results confirm those obtained by one of us on two previous patients.

We further investigated the point upon two rabbits inoculated respectively with sterilized cultures of cholera and typhoid.

The results of the blood examinations here made are subjoined in tabular form (Table VI.).

A comparison of the first and second testings of the cholera-inoculated rabbit² would seem to suggest that an initial reduction

¹ Wright, *Lancet*, Sept. 14, 1901, p. 715.

² The circumstance that a positive phase of increased bactericidal power was obtained in case of the typhoid rabbit without the intervention of a negative phase of diminished bactericidal power is in accordance with what occurs in man after the inoculation of a relatively small dose of typhoid vaccine (Wright, *Lancet*, Sept. 14, 1901, p. 715).

of the bactericidal power was exerted upon both species of micro-organisms. It would, in other words, seem to point to the comparability of the immediate effect exerted by the introduction of a sterilized culture of cholera into the animal organism with the effect exerted by the direct introduction of the culture into the serum *in vitro*.

On the other hand, a comparison of the results obtained in the first and last testings of both the typhoid and the cholera-inoculated rabbit will show that the increase of the bactericidal power which was achieved by inoculation was, in each case, an increase only with respect to the particular species of micro-organism which had been inoculated.

The latter datum is for our present purpose the essentially important one of the experiment. It seems to indicate clearly that the bactericidal effects of a serum, at any rate in the case of a serum derived from the immunized animal, is as is assumed by the theories of Ehrlich and Bordet respectively, achieved by the co-operation of two bactericidal elements, one of these being a chemical agent which exerts an action on more than one species of micro-organism, and the other a chemical agent which is specific for each particular species of micro-organism.

There is, however, nothing to forbid our explaining the bactericidal action of normal serum by the more simple assumption that the non-specific element referred to above ("complement" of Ehrlich, "alexin" of Bordet) suffices by itself to exert a bactericidal effect.

From the study of the action of the serum upon the typhoid bacillus and the cholera vibrio, we pass to the consideration of the action of the serum upon the *Staphylococcus pyogenes*.

II. *Data with regard to the bactericidal power of the blood as affecting the Staphylococcus pyogenes.*

As a preliminary to setting forth our results, we may observe that we have not in our numerous experiments found any difference of behaviour as between the different varieties of the *Staphylococcus pyogenes*. For this reason we have thought it unnecessary to encumber the tables given below by specifying in each case the particular variety of *Staphylococcus* employed. Suffice it to say that these were chiefly cultures of the *Staphylococcus aureus* and *albus* freshly cultivated from operation-wounds, furuncles and sycosis.

We set forth first a series of typical experiments conducted by mixing in capillary testing pipettes in each case one volume of serum

TABLE VII.

Exhibiting the results obtained on cultivating a mixture of equal volumes of serum and of a graduated dilution of Staphylococcus culture which had remained in contact for 18—24 hrs. at 37° C.

Dilutions in which the living Staphylococcus culture was employed	Capillary testing pipettes were filled with				
	1 vol. dilution of Staphylococcus culture and				
	1 vol. sterile broth	1 vol. F.N.W.'s serum	1 vol. A.E.W.'s serum	1 vol. W.B.L.'s serum	1 vol. J.A.'s serum
10-fold dilution	growth	growth	growth	growth	growth
100 " "	"	"	"	"	"
1000 " "	"	"	"	"	"
10,000 " "	"	"	"	"	"
100,000 " "	"	"	"	"	"
1,000,000 " "	"	"	"	"	"
10,000,000 " "	sterile	"	"	"	"

With regard to F.N.W., A.E.W., and the general conditions of the experiments see notes to Table I. W.B.L. was a man in normal health. J.A. had suffered from furunculosis and syosis barbae for a period of 9 years, and had completely recovered after three successive inoculations of a sterilized culture of a *Staphylococcus aureus* cultivated from his boils.

and one volume of a progressively increasing dilution of a 24-hour-old *Staphylococcus* culture.

It will be manifest that the results set forth in Table VII. are in conformity with the results obtained with the blood of animals in the classical researches of Nuttall¹. They show that human serum does not exert any bactericidal effect whatever upon the *Staphylococcus*; nay more, they suggest, and this suggestion is confirmed by direct observation on the colonies grown² in capillary testing pipettes filled with equal volumes of serum and gelatine cultures of *Staphylococcus*, that additions of serum exert a favourable influence on the growth of this germ.

Not obtaining any indication of a bactericidal effect exerted in the case of the volume for volume mixture of serum and broth dilutions of *Staphylococcus* cultures, we experimented further, using dilutions of broth cultures made with the serum under examination. In the higher dilutions thus obtained, we are in point of fact dealing with practically undiluted serum.

The method of experimentation adopted was as follows:—Two series of progressive dilutions of the culture were made, the diluents employed being in the one case sterile nutrient broth, and in the other case human serum.

A series of equal volumes of each dilution was measured off into capillary testing pipettes. These measured volumes were in the case of the broth dilutions immediately transferred to the surface of the nutrient agar with a view to the enumeration of the contained *Staphylococci*. The serum dilutions, on the contrary, were before implantation upon agar digested for 24 hours at 37° C. with a view to allowing the serum to exert its full effect upon the micro-organisms.

The results are set forth in Table VIII.

An arithmetical calculation based upon the data set forth in Table VIII., indicates that in the first experiment 10 c.mm. of practically undiluted serum failed to kill 0·4, and in the second experiment the same quantity of practically undiluted serum failed to kill 3 of the *Staphylococci* employed.

From the fact that the serum does not exert any bactericidal effect upon the *Staphylococci*, we surmised that no bactericidal substances

¹ *Zeitschrift f. Hygiene*, 1888, vol. iv. pp. 353—394.

² The technique employed in connection with the observations here in question was that which was described by one of us in the *Lancet*, Dec. 1, 1900, pp. 1556—1560.

TABLE VIII.

Exhibiting the results of the cultivations undertaken in the case of Staphylococcus cultures diluted (a) with sterile broth, and (b) with undiluted serum.

Dilutions of the cultures which were employed	6 day broth culture of Staphylococcus		2 day broth culture of Staphylococcus	
	diluted with sterile broth, then transferred to nutrient agar and incubated	diluted with A. E. W.'s serum, digested with this 24 hours at 37° C. and then cultivated in nutrient broth	diluted 1,000,000-fold with sterile broth, then transferred to nutrient agar and cultivated	diluted with A. E. W.'s serum, digested with this 24 hours at 37° C. and then cultivated in nutrient broth
10-fold dilut.		growth obtained from circ. 10 c.mm.		growth obtained from circ. 5 c.mm.
100 "		" "	9 colonies from 25 c.mm.	" "
1,000 "		" "	6 " 15 "	" "
10,000 "		" "	6 " 15 "	" "
100,000 "	40 colonies developed from 10 c.mm.	" "	2 " 10 "	" "
1,000,000 "	0 colonies developed from 20 c.mm.	" "	0 " 5 "	" "
10,000,000 "		" "	0 " 5 "	" "
		" "	" "	sterile
				growth obtained from circ. 5 c.mm.

M. G. who had been a martyr to furunculosis had recently undergone 3 successive therapeutic inoculations with sterilized Staphylococcus cultures.

TABLE IX.

Exhibiting the results obtained on adding a sterilized culture of Staphylococcus to a mixture of serum and living typhoid culture.

Dilutions in which the living typhoid culture was employed	Capillary testing pipettes were filled in with							
	1 vol. F. N. W.'s serum 1 vol. dilution of living typhoid culture		1 vol. A. E. W.'s serum 1 vol. dilution of living typhoid culture		1 vol. E. A. S.'s serum 1 vol. dilution of living typhoid culture		1 vol. W. G. L.'s serum 1 vol. dilution of living typhoid culture	
	1 vol. sterile broth	1 vol. sterilized Staphylococcus culture	1 vol. sterile broth	1 vol. sterilized Staphylococcus culture	1 vol. sterile broth	1 vol. sterilized Staphylococcus culture	1 vol. sterile broth	1 vol. sterilized Staphylococcus culture
2-fold dilut.	growth	growth	growth	growth	growth	growth	growth	growth
5 "	"	"	"	"	"	"	"	"
10 "	"	"	sterile	sterile	"	"	"	"
25 "	"	"	"	"	"	"	"	"
50 "	"	"	"	"	"	"	"	"
100 "	sterile	sterile	sterile	sterile	sterile	sterile	sterile	sterile
1000 "	"	"	"	"	"	"	"	"
10,000 "	"	"	"	"	"	"	sterile	"
100,000 "	"	"	"	"	"	"	"	sterile

E. A. S. was a man in normal health.

TABLE X.

Exhibiting the results obtained when a sterilized culture of Staphylococcus is added to a mixture of serum and living cholera culture.

Capillary testing pipettes were filled with								
Dilutions in which the living cholera culture was employed	1 vol. F. N. W.'s serum 1 vol. dilution of living cholera culture and		1 vol. A. E. W.'s serum 1 vol. dilution of living cholera culture and		1 vol. W. G. L.'s serum 1 vol. dilution of living cholera culture containing 2,600,000 cholera vibrios per c.c. and		1 vol. W. B. L.'s serum 1 vol. dilution of living cholera culture containing 21,500,000 cholera vibrios per c.c. and	
	1 vol. sterile broth	1 vol. sterilized Staphylococcus culture	1 vol. sterile broth	1 vol. sterilized Staphylococcus culture	1 vol. sterile broth	1 vol. sterilized Staphylococcus culture	1 vol. sterile broth	1 vol. sterilized Staphylococcus culture
undiluted	—	—	—	—	growth	growth	growth	growth
2-fold dilution	growth	growth	growth	growth	"	"	"	"
5 "	"	"	"	"	"	"	"	"
10 "	sterile	sterile	sterile	sterile	"	"	sterile	sterile
25 "	"	"	"	"	"	"	"	"
50 "	"	"	"	"	"	"	"	"
100 "	"	"	"	"	"	"	"	"
1000 "	"	"	"	"	"	"	"	"
10,000 "	"	"	"	"	sterile	sterile	"	"
100,000 "	"	"	"	"	"	"	"	"
"	—	—	—	—	"	"	"	"

would be extracted from the serum *in vitro* by the addition of a sterilized culture of *Staphylococcus*.

The substantial correctness of this inference was tested by means of the experiment set forth in Tables IX. and X. It must be noted that in these experiments we employed, not as in the experiments set forth in Tables I., III. and IV., a sterilized broth culture, but a very dense bacterial suspension made from one or more agar cultures.

It will be seen that with the exception of experiments 3, 4 and 5 in Table IX., where the difference is in each case a very small one, the bactericidal effect exerted was in no case less in the case of the serum which had received an addition of sterilized *Staphylococcus* culture than in the case of the serum which had received only an addition of sterile nutrient broth.

On reviewing the results obtained, we cannot fail to be struck with the sharp contrast between those obtained with the *Staphylococcus* and those obtained with the typhoid bacillus and cholera vibrio.

We have seen (a) that the typhoid bacillus and the cholera vibrio are killed off in very large numbers by the normal serum.

(b) That sterilized cultures of these micro-organisms when added to the serum *in vitro* extract from this last a bactericidal element.

(c) That the introduction of sterilized cultures of these bacteria into the human and animal organism, confers upon the animal an increased bactericidal power, with respect to the particular species of micro-organisms inoculated.

On the other hand, we have seen in the case of the *Staphylococcus* :

(a) That this micro-organism is favourably, rather than unfavourably, affected by contact with the normal serum.

(b) That sterilized cultures of this micro-organism added to the serum *in vitro* do not, unless possibly to a very small extent, diminish its bactericidal action upon the typhoid bacillus and the cholera vibrio.

Lastly, it would seem from the experiment in the last column of Table VIII. and from certain other observations which will be discussed elsewhere :

(c) That the introduction of sterilized cultures of the *Staphylococcus* into the human organism does not confer upon the serum any bactericidal power.

In view of the important bearing of facts such as those just disclosed in connection with the theory of immunity and in connection with protective inoculation, we now proceeded to draw within the scope of our enquiry, on the one hand, the *Bacillus pestis*, and on the other hand, the *Micrococcus melitensis*.

TABLE XI.

Exhibiting the results obtained by cultivating mixtures of one volume of a graduated dilution of a culture of the bacillus of plague and one volume of broth or of serum.

Capillary testing pipettes were filled with			
Dilutions in which the living plague culture was employed	1 vol. dilution of living plague culture (cultivated 4 days at 37° C.)		
	1 vol. sterile broth	1 vol. F. N. W.'s serum and	1 vol. A. E. W.'s serum
10-fold dilution	growth	growth	growth
25 "	"	"	"
50 "	"	"	"
100 "	"	"	"
1000 "	"	"	"
10,000 "	sterile	"	"
100,000 "	"	sterile	"

A. E. W. had undergone an inoculation with 'half a dose' of Haffkine's plague vaccine 2 years previously.

TABLE XII.

Exhibiting the results obtained by making graduated dilutions of a 2 day old living plague culture, with sterile broth and serum respectively; and incubating one, or in most cases two 10 c.mm. volumes of each dilution after transference to the surface of nutrient agar. This transference was in case of the serum dilution postponed for 24 hours. The capillary testing pipettes were in the interval kept at a temperature of 37° C.

Dilutions of the living plague culture which were employed	Number of colonies which developed on the nutrient agar in case of the			
	Dilution made with sterile broth	Dilution made with F. N. W.'s serum	Dilution made with A. E. W.'s serum	Dilution made with E. A. S.'s serum
1000-fold dilut.	60	innumerable	innumerable	innumerable
{10,000 " (a)	{12	{ " "	{ " "	{ " "
{10,000 " (b)	{14	{ " "	{ " "	{ " "
{100,000 " (a)	{ 0	{60	{40	{ " "
{100,000 " (b)	{ 2	{50	{35	{35
{1,000,000 " (a)	{ 1	{ 1	{ 1	{50
{1,000,000 " (b)	{ 1	{ 4	{ —	{50

With regard to A. E. W. see note to Table XI.

III. *Data with regard to the bactericidal power of the blood as affecting the Bacillus pestis.*

The observations recorded below suggest that, as in the case of the *Staphylococcus*, a favourable rather than an unfavourable influence is exerted upon the plague bacillus by human serum when mixed in equal volumes with a plague culture (see Table XI.).

The effect of the serum was further investigated by comparing the number of living plague colonies obtained from equal volumes of progressive dilutions of a plague culture made (a) with sterile nutrient broth, and (b) with human serum.

The results obtained are set forth in Table XII.

It will be manifest that the results bear testimony to the absence of a bactericidal effect and to a multiplication of the plague bacilli in almost all the serum tubes.

Following out the plan pursued in the case of the other micro-organisms treated of above, we now sought to determine whether any bactericidal element was extracted when a sterilized plague culture was added to a mixture of serum and living typhoid or living cholera culture. The method of investigation was the same as in the *Staphylococcus* experiments (Tables IX. and X.), a very dense bacterial suspension being made from one or more agar cultures. The results obtained are given in Tables XIII. and XIV.

It will be seen that the bactericidal power was practically unaffected by the addition of a sterilized plague culture.

IV. *Data with regard to the bactericidal power of the blood and the Micrococcus melitensis.*

The data obtained in the case of the Malta fever micrococcus hardly seem to require anything in the way of verbal comment. They are subjoined in the form of Tables XV. to XIX. inclusive.

Tables XV. and XVI. show that human serum, when mixed volume for volume with cultures of *Micrococcus melitensis*, is without action upon this micro-organism.

Table XVII. establishes that even the undiluted serum is entirely without bactericidal action, and that a multiplication of the micro-organism may take place in this medium.

Tables XVIII. and XIX. establish that the antibactericidal effect exerted by the addition of a dense suspension of *Micrococcus melitensis* upon human serum is quite insignificant.

TABLE XIII.

Exhibiting the results obtained on adding a sterilized culture of the plague bacillus to a mixture of serum and living culture of typhoid.

	Capillary testing pipettes were filled in with							
	1 vol. F. N. W.'s serum 1 vol. of a living typhoid culture containing 3000,000,000 <i>T.E.</i> per c.c. and	1 vol. A. E. W.'s serum 1 vol. of a living typhoid culture containing 3000,000,000 <i>T.E.</i> per c.c. and	1 vol. F. N. W.'s serum 1 vol. of a living typhoid culture and	1 vol. A. E. W.'s serum 1 vol. of a living typhoid culture containing 1100,000,000 <i>T.E.</i> per c.c. and	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth
Dilutions of the living typhoid culture which were employed	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth	1 vol. sterile broth
undiluted culture	growth	growth	growth	growth	growth	growth	growth	growth
2-fold dilution	"	"	"	"	"	"	"	"
5 "	"	"	"	"	"	"	"	"
10 "	"	"	"	"	"	"	"	"
25 "	"	"	"	"	"	"	"	"
50 "	"	"	"	"	"	"	"	"
100 "	"	"	"	"	"	"	"	"
1000 "	"	"	"	"	"	"	"	"
10,000 "	"	"	"	"	"	"	"	"
100,000 "	"	"	"	"	"	"	"	"

* The irregularity was probably due to an accidental overheating of the tube.

Bactericidal Effects of Blood

TABLE XIV.

Exhibiting the results obtained by the addition of a sterilized plague culture to a mixture of serum and living culture of cholera.

Dilutions in which the living culture of cholera was employed	Capillary testing pipettes were filled in with							
	1 vol. F. N. W.'s serum 1 vol. living cholera culture containing 18,000,000 chol. vibrios per c.c. and		1 vol. A. E. W.'s serum 1 vol. living cholera culture containing 18,000,000 chol. vibrios per c.c. and		1 vol. F. N. W.'s serum 1 vol. living cholera culture containing 44,000,000 chol. vibrios per c.c. and		1 vol. A. E. W.'s serum 1 vol. living cholera culture containing 44,000,000 chol. vibrios per c.c. and	
	1 vol. sterile broth	1 vol. sterilized plague culture	1 vol. sterile broth	1 vol. sterilized plague culture	1 vol. sterile broth	1 vol. sterilized plague culture	1 vol. sterile broth	1 vol. sterilized plague culture
undiluted culture	growth	growth	growth	growth	growth	growth	growth	growth
2-fold dilution	sterile	sterile	sterile	sterile	"	"	"	"
5 "	"	"	"	"	"	"	"	"
10 "	"	"	"	"	"	"	"	"
25 "	"	"	"	"	"	"	sterile	sterile
50 "	"	"	"	"	"	"	"	"
100 "	"	"	"	"	"	"	"	"
1000 "	"	"	"	"	"	"	"	"
10,000 "	"	"	"	"	"	"	"	"

TABLE XV.

Exhibiting the results obtained on cultivating equal volumes of serum and diluted Micrococcus meliensis culture which had remained in contact at 37°C. for 24 hours.

Dilutions in which the living Malta fever culture was employed	Capillary testing pipettes were filled in with				
	1 vol. of a 4 day old broth culture of <i>Micrococcus meliensis</i>				
	1 vol. sterile broth	1 vol. F. N. W.'s serum	1 vol. W. B. L.'s serum and 1 vol. A. B.'s serum	1 vol. J. W.'s serum	
10-fold dilution	growth	growth	growth	growth	growth
25 "	"	"	"	"	"
50 "	"	"	"	"	"
100 "	"	"	"	"	"
1000 "	"	"	"	"	"
10,000 "	"	"	"	"	"
100,000 "	"	"	"	"	"
1,000,000 "	sterile	sterile	sterile	sterile	sterile

TABLE XVI.

Exhibiting the results obtained by cultivating at 25° C. in capillary tubes equal volumes of serum and diluted gelatine culture of the Micrococcus melitensis.

Dilutions of the gelatine culture which were employed	Capillary tubes were filled in with 1 volume of the culture of <i>Micrococcus melitensis</i> diluted with nutrient gelatine (15% gelatine) and		
	1 vol. sterile broth	1 vol. F. N. W.'s serum	1 vol. A. E. W.'s serum
	no. of colonies which developed in the tubes	no. of colonies which developed in the tubes	no. of colonies which developed in the tubes
10-fold dilution	innumerable	innumerable	innumerable
100 "	"	"	"
1000 "	100 (circ.)	100 (circ.)	100 (circ.)
10,000 "	17	20	14
100,000 "	15	23	20
1,000,000 "	3	6	4

The tubes were filled in and the colonies in the capillary tubes were counted under the microscope by the technique described by one of us in the *Lancet*, Dec. 1st 1900, pp. 1556—1560.

TABLE XVII.

Exhibiting the results obtained by diluting 4 day old culture of the Micrococcus melitensis with sterile broth and human serum respectively, and by cultivating 10 c.mm., or, where specified, 5 c.mm., of each dilution on nutrient agar. The transference to nutrient agar was in the case of the serum dilutions postponed for 24 hrs. During this interval the capillary testing pipettes were kept at 37° C.

Dilutions in which the culture was employed	Number of colonies which developed in the case of the			
	Dilutions of culture no. 1 made with sterile broth	Dilutions of culture no. 1 made with F. N. W.'s serum	Dilutions of culture no. 2 made with sterile broth	Dilutions of culture no. 2 made with A. E. W.'s serum
100-fold dilution				
1000 "	—	innumerable	—	—
" "	—	"	—	—
{10,000 (a)	{innumerable	{"	{"	{innumerable
{10,000 (b)	{"	{"	{"	{"
{100,000 (a)	{innumerable	{"	{113	{100 (circ.)
{100,000 (b)	{"	{"	{68	{"
{1,000,000 (a)	{50	{"	{15	{50 (5 c.mm.)
{1,000,000 (b)	{1	{80	{1	{"
10,000,000 "	—	—	—	—

TABLE XVIII.

Exhibiting the results obtained by the addition of a sterilized dense suspension of the Micrococcus melitenensis to a mixture of serum and living typhoid culture.

Dilutions in which the living typhoid culture was employed	Capillary testing pipettes were filled in with							
	1 vol. living typhoid culture and 1 vol. F.N.W.'s serum		1 vol. living typhoid culture and 1 vol. E.A.S.'s serum		1 vol. living typhoid culture and 1 vol. W.H.L.'s serum		1 vol. living typhoid culture and 1 vol. F.N.W.'s serum	
	1 vol. sterile broth	1 vol. sterilized M.m. culture	1 vol. sterile broth	1 vol. sterilized M.m. culture	1 vol. sterile broth	1 vol. sterilized M.m. culture	1 vol. sterile broth	1 vol. sterilized M.m. culture
undiluted culture	growth	growth	growth	growth	growth	growth	growth	growth
2-fold dilut.	"	"	"	"	"	"	"	"
5 "	"	"	"	"	"	"	"	"
10 "	sterile	"	"	"	"	"	"	"
25 "	"	"	"	"	"	"	"	"
50 "	"	"	"	"	"	"	"	"
100 "	"	"	sterile	"	"	"	"	"
1000 "	"	"	"	"	"	"	"	"
10,000 "	"	sterile	"	"	"	sterile	"	sterile
100,000 "	"	"	"	"	"	"	"	"

TABLE XIX.

Exhibiting the results obtained by the addition of a sterilized dense suspension of the Micrococcus melioides to a mixture of serum and living cholera culture.

Dilutions in which living cholera culture was employed	Capillary testing pipettes were filled with							
	1 vol. living cholera culture and 1 vol. E.A.S.'s serum		1 vol. living chol. culture containing 18,000,000 c. vibrios per c.c. 1 vol. F.N.W.'s serum		1 vol. living chol. culture containing 44,000,000 c. vibrios per c.c. 1 vol. F.N.W.'s serum		1 vol. living chol. culture containing 44,000,000 c. vibrios per c.c. 1 vol. A.E.W.'s serum and	
	1 vol. sterile broth	1 vol. sterilized M.m. culture	1 vol. sterile broth	1 vol. sterilized M.m. culture	1 vol. sterile broth	1 vol. sterilized M.m. culture	1 vol. sterile broth	1 vol. sterilized M.m. culture
undiluted culture	growth	growth	growth	growth	growth	growth	growth	growth
2-fold dilut.	"	"	sterile	"	"	"	"	"
5 "	"	"	"	"	"	"	"	"
10 "	"	"	"	"	"	"	"	"
25 "	"	"	"	"	"	"	"	"
50 "	sterile	sterile	"	"	"	sterile	"	"
100 "	"	"	"	"	"	"	"	"
1000 "	"	"	"	"	"	"	"	"
10,000 "	"	"	"	"	"	"	"	"
100,000 "	"	"	"	"	"	"	"	"

CONCLUSIONS.

On reviewing the experimental data which we have set forth, it would seem clear that—

(1) Human serum has a powerful bactericidal effect upon the typhoid bacillus, and the cholera vibrio, while it is without bactericidal action upon the *Staphylococcus pyogenes*, *B. pestis*, *Micrococcus meli-tensis* (and so far as we have gone, upon the *Streptococcus pyogenes*, and *B. diphtheriae*).

(2) Sterilized cultures of those species of pathogenic micro-organisms which are killed by the serum, appear, in contradistinction to those species of micro-organisms which are not affected by the serum, to possess the power of directly abstracting a bactericidal element from the blood.

The first of these generalizations appears to possess a far-reaching significance in connection with the general theory of immunity.

(a) It has an obvious bearing on the question of the mechanism by which bacteria are destroyed in the organism.

(b) It also bears on the question as to whether the bactericidal action is acquired only after withdrawal from the organism, and after the disintegration of leucocytes.

For it would seem difficult to assume that the bactericidal power of the serum is only a particular manifestation of a digestive power or originally resident in the leucocyte, when we have realized that the serum exerts a bactericidal action only on particular species of micro-organisms while the leucocyte exerts a digestive action on bacteria generally.

The second of the generalizations arrived at above would seem to point to the bactericidal effects being the result of definite chemical combinations occurring between the bactericidal substance or substances in the blood and the affected bacteria.

In conclusion, reference may be made to a possible relation between the danger or relative absence of danger associated with the hypodermic inoculation of different species of bacteria, and the effect or absence of effect of the blood upon these micro-organisms. A notable contrast obtains in this respect between the event of inoculations of cholera and typhoid on the one hand, and plague and Malta fever on the other hand.

While inoculation with living cultures of cholera is, as has been

shown in connection with Haffkine's anticholera inoculations, practically unassociated with risk, and while inoculations with small quantities of living typhoid bacilli are—judging from the event of an experimental inoculation undertaken by one of us, and from the immunity from accident which has attended wholesale manipulations with this micro-organism—associated with only slight risk, the results are quite other in the case of even minimal inoculations of plague and Malta fever cultures.

That extreme risk attaches to the inoculation of even minimal quantities of living plague bacilli is attested by the numerous cases of plague which have supervened upon the accidental inoculation of infected material into small superficial scratches.

The risk attaching to even minimal inoculations of the *Micrococcus melitensis* is less well known. Six cases of the disease have occurred in connection with bacteriological work on Malta fever undertaken at Netley, and two further cases have originated at the Royal Naval Hospital, Haslar, and in the Philippines respectively, in connection with bacteriological work.

Of the cases occurring at Netley, one originated from an accidental prick with a needle of a syringe containing a Malta fever culture; a second arose in connection with an experimental inoculation; and a third has recently occurred in connection with the accidental projection of the end of a contaminated capillary sedimentation tube into the eye. The three other cases at Netley arose apart from a recognized inoculation in the case of observers working with living cultures. It would seem difficult to conceive of inoculations with quite minimal quantities of cultures being so effectual in the case of micro-organisms subject to the bactericidal action of the blood and lymph.

THE AIR OF FACTORIES AND WORKSHOPS.

By JOHN S. HALDANE, M.D., F.R.S.

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By the existing law for Great Britain it is enacted that "in every room in any factory or workshop sufficient means of ventilation shall be provided, and sufficient ventilation shall be maintained." Not very much has been hitherto ascertained, however, as to the degree of purity actually existing in the air of factories and workshops generally, or as to what standard of purity may reasonably be expected considering the difficulties met with. A Departmental Committee was recently appointed by the Home Secretary to investigate and report upon the subject. The members of the Committee were Mr E. H. Osborn, Engineering Adviser to the Chief Inspector of Factories, and myself, with Mr C. R. Pendock, one of H. M. Inspectors of Factories, as Secretary. The Committee has just reported on the subject of general ventilation¹, but reserving for a future Report the vitiation of air by dust, fumes, &c. from special manufacturing processes.

In the present paper I propose to give an account of the air of factories and workshops in the light of the facts ascertained by the Committee in the course of their experimental investigations on general ventilation.

¹ *Report of the Departmental Committee on Factory Ventilation, Parliamentary Paper, 1902.*

Methods of Analysis.

Throughout the investigation the proportion of CO_2 in the air was mainly relied on as a measure of the impurity of the air. Although the slight excess of CO_2 (together with the corresponding deficiency in oxygen) in the air of inhabited rooms is in itself of no importance, it is certainly the simplest and most certain objective index of the probable proportion of those other impurities which cause air contaminated by persons and lights to be unwholesome.

The bleaching action of the air on permanganate solution has also been sometimes used as an index, but this method was not employed, as its significance is very uncertain, particularly as even in badly ventilated rooms most of the bleaching action is evidently due to smoke &c. present in ever-varying proportion in the outside air of towns. Even undiluted expired air has only a very slight bleaching action. As an average of twelve experiments the *excess* in bleaching action of expired air over inspired air was found to be less than the average bleaching action of outside air in Dundee¹.

To supplement the information obtained from CO_2 determinations the number of micro-organisms per litre of air was ascertained by us in a certain proportion of samples. When the number in outside air is small, as in winter or wet summer weather, this method gives results of some value, as where the amount of physical disturbance does not greatly differ in different rooms the number of bacteria in the air is a fair index of general cleanliness, and consequently of the probability of pathogenic bacteria being present. In many factories, however, large numbers of bacteria are present in the materials employed, and unless there is any reason to suppose that these materials may contain pathogenic bacteria not much importance can be attributed to the mere number of bacteria in a given volume of air.

The method employed for determining carbonic acid was described by me in this *Journal*, Vol. I. p. 109. The chief advantages of this method are, (1) that the analysis can be accurately carried out on the spot within less than five minutes; (2) that when samples of the air are collected for analysis at a more convenient time very small bottles are sufficient, and may be filled without loss of time. A bottle of 50 c.c. capacity permits of a double analysis being made.

To reduce the size of the apparatus, and render it more convenient, some modifications in the original apparatus were introduced: (1) The

¹ Carnelley, Haldane, and Anderson, *Philosophical Transactions*, 1887, B. p. 87.

vessel within the case of the apparatus for holding the water used as a confining liquid in the analysis of bottle samples was dispensed with. An ordinary tumbler supported outside the case serves equally well if water is used. (2) The mercury reservoir was arranged so as to be supported by its neck from the front of the lower shelf of the case. With the saving of space thus effected the size of the case (internal measurements) was reduced to $12 \times 6\frac{1}{2} \times 2\frac{1}{2}$ inches¹.

For the collection of samples of air in bottles it was found best to employ dry and clean glass-stoppered bottles of about 50 c.c. capacity, as experience showed that bottles with ordinary corks coated with paraffin, as originally recommended, were not sufficiently tight if the sample was kept for long. The stopper of each bottle was lubricated with vaseline and held in position by an elastic band passing vertically round the bottle. A gummed label passing round the bottle over the elastic band secured the latter more firmly. On inserting the stopper it was turned round so that no air-channels were left in the vaseline.

In order to test this method of keeping samples the following experiments were made. The bottles used were of about 65 c.c. capacity.

I. Four samples of outside air (country) collected simultaneously in dry and clean bottles were kept for varying periods and then analysed.

				Vols. per 10,000.
Bottle 1.	Analysed at once	...		{(a) 3.0
				{(b) 3.0
" 2.	"	after 5 days		2.8
" 3.	"	" 9 "		3.0
" 4.	"	" 19 "		{(a) 2.8
				{(b) 3.0

II. Five samples of air collected simultaneously in a room containing vitiated air were similarly kept and analysed.

				Vols. per 10,000.
Bottle 1.	Analysed at once	...		{(a) 51.0
				{(b) 51.4
				{(c) 50.8
" 5.	"	" " "	...	50.8
" 2.	"	after 2 days		51.2
" 3.	"	" 6 "		{(a) 50.7
				{(b) 51.0
" 4.	"	" 14 "		{(a) 50.6
				{(b) 50.4

¹ A detailed description of all the apparatus used is given in Appendix III. of the Report. The apparatus may be obtained from Messrs Müller, Orme, and Co., 148, High Holborn, London.

These experiments show that the carbonic acid does not increase or diminish within the bottles to an appreciable extent within a period much longer than would be required if the samples were sent away for analysis.

To test the effects produced by the bottles being wet or dirty the following additional experiments were made.

III. Four samples of outside air were collected in clean but wet bottles.

				Vols. per 10,000.
Bottle 1.	Analysed at once	...		3.0
" 2.	"	after 3 days	$\left\{ \begin{array}{l} (a) \\ (b) \end{array} \right.$	$\left\{ \begin{array}{l} 2.4 \\ 2.2 \end{array} \right.$
" 3.	"	" 5 "	$\left\{ \begin{array}{l} (a) \\ (b) \end{array} \right.$	$\left\{ \begin{array}{l} 2.0 \\ 2.0 \end{array} \right.$
" 4.	"	" 12 "	$\left\{ \begin{array}{l} (a) \\ (b) \end{array} \right.$	$\left\{ \begin{array}{l} 0.2 \\ 0.0 \end{array} \right.$

IV. Four samples of vitiated air were collected simultaneously, two being in clean and dry bottles, and two in clean and wet bottles.

				Vols. per 10,000.
Bottle 1 (dry).	Analysed at once	...	$\left\{ \begin{array}{l} (a) \\ (b) \end{array} \right.$	$\left\{ \begin{array}{l} 20.6 \\ 20.8 \end{array} \right.$
" 3 (dry).	"	after 9 days	$\left\{ \begin{array}{l} (a) \\ (b) \end{array} \right.$	$\left\{ \begin{array}{l} 21.2 \\ 21.2 \end{array} \right.$
" 2 (wet).	"	at once	$\left\{ \begin{array}{l} (a) \\ (b) \end{array} \right.$	$\left\{ \begin{array}{l} 21.0 \\ 20.4 \end{array} \right.$
" 4 (wet).	"	after 9 days	$\left\{ \begin{array}{l} (a) \\ (b) \end{array} \right.$	$\left\{ \begin{array}{l} 18.6 \\ 18.6 \end{array} \right.$

The last two experiments show that in bottles which were clean, but wet, a slow absorption of carbonic acid occurred. This was probably due to the presence of alkali dissolved by the water from the glass.

V. Five samples of outside air were collected in bottles which were dry, but very dirty from dust introduced.

				Vols. per 10,000.
Bottle 1.	Analysed at once	...		3.0
" 2.	"	after 3 days		3.2
" 3.	"	" 5 "	$\left\{ \begin{array}{l} (a) \\ (b) \end{array} \right.$	$\left\{ \begin{array}{l} 3.2 \\ 3.0 \end{array} \right.$
" 4.	"	" 12 "		3.0
" 5.	"	" 17 "		2.8

VI. Four samples of outside air were collected simultaneously in bottles which were both wet and very dirty from dust introduced.

				Vols. per 10,000.
Bottle 1.	Analysed	at once	...	3.0
" 2.	"	after 2 days		2.8
" 3.	"	"	6 "	{(a) 8.0
				{(b) 7.6
" 4.	"	"	12 "	15.0

The last experiment shows that in bottles which are both wet and dirty the carbonic acid may increase very considerably within a short period.

The bottles used should be cleaned with a brush and water, and afterwards rinsed with distilled water and dried by heating. If alcohol and ether are used for drying very great care must be taken to remove the last traces of ether. Bottles used a second time require a fresh coating of vaseline round the stopper.

For the analysis of bottle samples mercury was used as a confining liquid. The stopper was removed under mercury in a small mortar, and the bottle, with its mouth closed by a finger, transferred to a trough similar to that described by me at p. 477, Vol. XXII. of the *Journal of Physiology*. The sample for analysis was then withdrawn through a curved tube into the air-burette. As, however, the sample was withdrawn at negative pressure, the mercury reservoir of the apparatus was depressed below the level of the table before the tap was closed, so that the pressure in the burette was positive when the reservoir was replaced on its hook. The excess of air was then let out by opening to the air an extra three-way tap inserted between the opening of the burette and the curved tube communicating with the bottle. The analysis could then be carried out without further trouble.

If water is used as the confining liquid only one analysis can be made from each bottle, as contact with water alters the proportion of carbonic acid after a short time.

For the determination of bacteria in air the method of Frankland¹, with a few slight modifications, was employed. According to this method a measured quantity of air is drawn through a sterile glass tube containing a plug of glass-wool or similar material. The brass syringe used for aspirating also measures the air. This plug, which arrests all the bacteria, is afterwards pushed out into a flask containing a small

¹ *Philosophical Transactions*, 1887, B. p. 113.

amount of Koch's nutrient jelly, previously sterilised, in which, when liquefied, the plug is disintegrated by shaking. The jelly is then cooled and allowed to become solid in a thin layer on the sides of the flask, which is kept at a temperature of about 20° till no more colonies develop. The modifications introduced were chiefly with a view to convenience. The glass tubes were shorter and somewhat narrower than those described by Frankland, and a second control plug was dispensed with, as Frankland's experiments showed it to be unnecessary. Each tube was sterilised in a separate outer tube closed by an asbestos plug. This was a great convenience, as the tubes could be carried in a cigar-case, and the handling of them was much simplified. The inner tubes were fixed directly by means of a junction of stout rubber to the brass syringe, the mercury gauge employed by Frankland being found unnecessary. Everything needed was thus very easily carried about. Finally, flat-bottomed bacteriological flasks were used for the jelly, instead of the ordinary flasks employed by Frankland. In this way the inconvenience due to liquefying colonies was greatly reduced. To facilitate the disintegration of the glass-wool plug the latter was crushed with a sterile glass rod against the bottom of the flask before the liquefied jelly was distributed.

Arrangement of Rooms and Ventilation in Factories.

In factories and workshops almost every variety in size and construction of rooms is met with; and the methods of ventilation, whether designed or accidental, vary correspondingly. As regards size the rooms which we visited varied from about 500 or 1000 cubic feet, as in small workrooms containing only two or three persons, to over 1,000,000 cubic feet with 1000 persons or more, as in some of the larger weaving or engineering sheds. In construction also the rooms varied greatly. They might be mere outhouses with lean-to roofs, as in hand file-cutting at Sheffield: or ordinary small rooms in buildings of several storeys, as in many tailoring workshops, &c.: or large rooms occupying the whole of one storey, and either communicating freely by inside stairs or lifts with rooms above and below, or isolated from them: or still larger rooms occupying the whole of a high building, and with galleries running round inside: or large sheds lighted from above. The roofs and walls also varied greatly as regards their permeability to air.

Sometimes there were no evident openings for ventilation, although we occasionally found that in such rooms the actual ventilation was

fairly good, on account of the permeability of the roof and walls : often there were open windows, and often other ventilating openings of various kinds, including open stairs, lift-shafts, and gratings to the rooms above or below. In the larger rooms ventilation by fans was found to be common, while in the smallest rooms open fireplaces often enough served as the chief means of ventilation during the colder weather.

The means of heating employed also varied greatly. In the larger rooms heating by steam-pipes, carried either near the floor, or overhead, was the commonest method. In smaller rooms gas-stoves usually provided with flues, ordinary coal-stoves, and open fires were employed. In many rooms, however, the heating arrangements were inadequate in cold weather, and the objectionable method of attempting to heat the room by lighting the ordinary gas-jets was often resorted to. Sometimes there was no other method of heating. Warming the incoming air was seldom resorted to, unless the air was artificially humidified, as in many cotton-cloth weaving sheds. In employments not of a sedentary nature heating arrangements were often not provided because they were not required. In other employments heat from the machines used was sufficient to warm the room. In certain employments, such as cotton-spinning, the nature of the work necessitated a very high temperature, which was maintained by steam-pipes and heat from the machines. By the Factory Act it is provided that "in every factory or workshop adequate means must be taken for securing and maintaining a reasonable temperature in each room in which any person is employed, but the means so taken must not interfere with the purity of the air of any room in which any person is employed." In sedentary occupations a temperature of not less than about 60° F. (15·5° C.) appears to be necessary for comfort.

As a general rule factories and workshops are not nearly so densely occupied as many ordinary public buildings, such as churches, theatres, schools, halls for public meetings, &c. This is due chiefly to the fact that considerable floor-space is needed in almost all employments, and partly also to the provision in the Factory Act that there shall be a minimum of 250 cubic feet of space to each person employed in any room, and 400 cubic feet during work overtime. The average cubic space per person in the rooms which we examined was 1875, or if rooms with over 5000 cubic feet per person be excluded, 925. In elementary schools Carnelley, Haldane, and Anderson¹ found an average of 168

¹ *Loc. cit.*

cubic feet; and in many public buildings the space per person is no greater. The relatively greater cubic space in factories and workshops renders their proper ventilation not so difficult as in the case of public buildings. Ventilation by natural means is usually more practicable, as there is not the same necessity for warming the incoming air in order to prevent unpleasant draughts, relatively gentle air-currents being sufficient.

Carbonic Acid in Outside Air.

Before discussing the average results of the analyses it is necessary to refer shortly to the variations in the carbonic acid of outside air. In the air of the open country the proportion of carbonic acid averages almost exactly 3·0 volumes per 10,000. The very careful and complete series of determinations made in France by Reiset¹ in 1872–80 gave an average for day and night of 2·96 volumes. He absorbed the carbonic acid with baryta water, and used 525 litres of air for each determination, of which there were 220. The following table shows the corrected results of a series of exact determinations by Miss E. S. Haldane and myself, made at Cloanden, Perthshire, in 1889–90, and not hitherto published. The method used was the gravimetric one of Haldane and Pembrey². The samples were taken at 4 feet from the ground, and 76·7 litres of air were used for each determination.

	No. of analyses	Volumes of CO ₂ per 10,000		
		Maximum	Minimum	Average
April 1—Sept. 30	Day	3·11	2·58	2·88
	Night	3·55	2·82	3·08
December—January	Day	3·12	2·93	2·99
	Night	3·06	2·94	3·01

The older determinations by Pettenkofer's method gave results which varied considerably according to the particular manipulations employed, and were usually too high by about 0·5 volumes per 10,000, though occasionally also too low. The action of the baryta water on the glass bottles probably accounts, in part at least, for the errors.

¹ *Annales de Chimie et de Physique*, Vol. xxvi. 1882, p. 198.

² *Philosophical Magazine*, 1890, p. 306.

The experiments of Angus Smith and others have shown that in English towns the proportion of carbonic acid in the outside air is sensibly greater than in the country. The most complete series of determinations is that of Dr Russell for London air at St Bartholomew's Hospital¹. Excluding foggy days his averages were as follows.

	No. of Analyses	Volumes of CO ₂ per 10,000		
		Maximum	Minimum	Average
April—September	92	4·8	3·0	3·81
October—March	40	6·4	3·2	4·22
				4·01

The average of 29 determinations on foggy days was 7·2 volumes, the maximum being 14·1 volumes and the minimum 4·5 volumes.

The method used was that of Pettenkofer, so that probably the average results were about 0·5 volumes too high.

Russell's experiments show clearly that on foggy days in towns the proportion of carbonic acid in the air inside a building may be considerably raised in consequence of the vitiated state of the outside air.

Average Results of Analyses in Factories.

The general average of carbonic acid in the rooms which we examined was 10·1 volumes per 10,000 for analyses made during day-light or with electric lighting, and 17·6 during gas-light. It should be remarked, however, that very few analyses were made during summer weather, when ventilation is usually much more free, owing to the opening of windows: also that the rooms visited were chiefly those in which without due care the air would be liable to become considerably vitiated. The average for *all* factories and workshops would doubtless be lower than the figures just given.

As the great majority of the rooms examined were in towns, with a probable average proportion of about 3·5 volumes per 10,000 in the outside air, the average excess of carbonic acid in the rooms examined was about 6·6 volumes with no gas burning and 14·1 volumes with gas burning. The carbonic acid in the outside air was actually determined in all cases where there was any mist or fog. The maximum proportion found was 6·5 volumes (during a fog in the City, London), and

¹ *St Bartholomew's Hospital Reports*, Vol. xx.

the average for such days and nights was 5·0 volumes, and on other days 3·4 volumes. The results of each analysis, both of outside and inside air, are contained in Appendix I. of the Committee's Report. All the analyses were made by the rapid method already referred to, which has been found to give an average result of 3·0 volumes for unvitiated outside air.

The maximum proportion of carbonic acid found by day was 46·2 volumes. This was in a very tightly-closed spinning room, with an average cubic space per person of 10,169 cubic feet. Gas was burnt in this room in the morning and evening, and the carbonic acid was undoubtedly mostly produced by combustion of gas many hours previously. The temperature was 33·3° (92° F.).

The average proportion of carbonic acid found is less than that in many public buildings, and evidently the air of factories and workshops generally is not relatively speaking so much vitiated by overcrowding as is sometimes supposed. To make only one comparison, the average proportion of carbonic acid in elementary schools (in Dundee) was found by Carnelley, Haldane, and Anderson to be 18·6 volumes per 10,000 with natural ventilation, and 12·3 volumes with the very imperfect mechanical ventilation employed at the time (1886) in some of the schools investigated. As a general rule employers, and particularly the more energetic and prosperous ones, are anxious to do all in their power to secure satisfactory ventilation. Bad ventilation is frequently due to objections on the part of a few of the employees, or to failure on the part of architects or others to carry out the intentions of employers. In a good many cases, however, far too much reliance is placed upon the existence of a large air-space per person employed.

Only about 40 determinations were made of micro-organisms. The average number per litre in rooms where there was no undue disturbance of dusty material (as occurs, for instance, in the preparation of cotton, jute, hemp, &c. for spinning) was 8·0 bacteria and 2·2 moulds, or 10·2 micro-organisms in all. The determinations from which this average is calculated were made chiefly in printers', bookbinders', tailors', and milliners' workrooms during the winter months, and indicate a fairly satisfactory standard of cleanliness. The average compares very favourably with the averages of 152 in elementary schools in Dundee¹, 76 in country board-schools in Scotland², 60 for

¹ Carnelley, Haldane, and Anderson, *loc. cit.*

² Carnelley and Foggie, *Journ. of Pathol. and Bacteriol.* Vol. II. p. 157.

one-roomed dwellings in Dundee¹, 46 for two-roomed dwellings¹, and 9 for the better classes of dwelling¹. No determinations were made by the Committee of micro-organisms in outside air, but in Dundee the average in the winter months was 0·8 per litre¹. In summer, as shown by Frankland² and others, the numbers in outside air in towns are far higher. By the first section of the Factory Act it is enacted that "every factory must be kept in a cleanly state."

In factories where much organic dust passes into the air from machines, &c., the number of micro-organisms in the air may of course be very great. Thus in a rope factory close to a dusty machine we found as many as 850 per litre. There was no reason to suspect, however, that any of the organisms present were pathogenic.

Influence of Combustion of Gas.

Were there no other products of the combustion of coal-gas except carbonic acid and moisture, the changes produced in the air of rooms by its combustion would be of little practical importance apart from the rise of temperature. Coal-gas, however, always contains a little sulphur—chiefly in the form of carbon bisulphide. This is burnt to sulphuric acid, which is apparently the cause of the characteristic oppressiveness of air much vitiated by the burning of gas. Air vitiated by the combustion of gas to the extent of 20 volumes per 10,000 begins to feel distinctly oppressive, even with well-purified gas. With good and clean paraffin lamps burning in a closed room I was unable to observe any similar effect even when the proportion of carbonic had risen as high as 75 volumes per 10,000.

The quantity of sulphur present in gas varies considerably in different towns, according as the gas is or is not thoroughly purified. In London, where the purification is good, and there is a legal limit to the amount of sulphur permitted, about 0·75 gramme of sulphur per 100 cubic feet of gas is usually present; but in some of even the larger English towns the amount of sulphur present is a good deal higher, so that air vitiated by combustion of gas is correspondingly more unpleasant.

A common gas-jet, such as is usually met with at present in English factories and workshops, consumes from 5 to 10 cubic feet of gas per hour; and this amount of ordinary English gas produces in burning from 2½ to

¹ Carnelley, Haldane, and Anderson, *loc. cit.*

² *Loc. cit.*

5 cubic feet of carbonic acid. The mean of two analyses which I made of ordinary gas of 16 to 17 candle-power gave the following results per volume of gas burnt.

Carbonic acid formed ...	0·54	volume.
Aqueous vapour „ ...	1·19	„
Oxygen consumed ...	1·14	„

As the aqueous vapour does not under ordinary circumstances condense, the products of combustion are, even after cooling, lighter than air: for although the carbonic acid is about 37 % heavier than the oxygen which it replaces, the aqueous vapour is about 42 % lighter, and present in much greater volume. From this circumstance and the fact that the heated products of combustion ascend in a concentrated stream towards the roof, and that gas-jets are usually at a height of six feet or more, the circulation of vitiated air from gas-jets is to a large extent above the breathing level. The following analyses of the air of a room of 5700 cubic feet and 11½ feet high illustrate this point. All openings were closed and only one person was present. Three No. 4 union burners were lit, passing in all about 15 cubic feet of gas per hour. The gas-jets were at a height of 6½ feet from the floor, on the walls at opposite sides of the room. The samples were taken at the centre of the room.

	Volumes of CO ₂ per 10,000		
	At 1 foot from floor	At 4 feet from floor	At 1 foot from roof
Before gas lit		2·9	
13 minutes after gas lit			13·8
18 „ „ „	7·5	4·8	
24 „ „ „			
30 „ „ „			20·2
36 „ „ „	12·7	9·0	
44 „ „ „			
53 „ „ „		13·9	
59 „ „ „	16·5		27·7
64 „ „ „			
68 „ „ „		17·4	
90 „ „ „	19·6		34·3
96 „ „ „		19·4	
102 „ „ „			
140 „ „ „	24·8		39·0
144 „ „ „		25·8	
148 „ „ „			

The temperature at the beginning of the experiment was 12.5° , and at the end 15.4° at 4 feet from the floor, and 19.4° at 1 foot from the roof. The outside temperature was 9° .

With the products of respiration the distribution in a room is different. In expired air about 4% of oxygen is replaced by about $3\frac{1}{2}\%$ of carbonic acid, and about 4 to 5% of aqueous vapour is added. This mixture, when diluted and cooled, is very nearly as heavy as pure air: it is not nearly so much heated as the products of combustion; and convection currents due to warming of the air by the bodies of the persons present cause it to mix very completely with the air of the room unless it can escape promptly at the roof. This is illustrated by the following experiment made in a room 11 feet high, and with 3070 cubic feet capacity. Four persons were present, and all openings closed. The samples were taken at the centre of the room.

	Volumes of CO ₂ per 10,000	
	At 4 feet from floor	At roof
Before experiment	2.8	
After 20 minutes	5.5	4.7
" 70 "	10.9	11.5
" 90 "	12.8	12.1
" 110 "	16.4	16.4
" 125 "	16.6	16.7
Average	10.37	10.23

In calculating the probable effect of combustion of gas on the purity of the air of a room it is evidently necessary to consider to what extent the arrangements for ventilation permit the heated and vitiated air from gas-jets to escape without mixing with the air at the breathing level. The experiment already quoted shows that in high rooms the air at the breathing level will usually be less vitiated by gas than in low rooms of equal cubic space. Where, however, the incoming air is introduced at a high level, or where driving-belts for machinery are constantly mixing the air at different levels, as in weaving-sheds, there is much more complete mixture than in other rooms, so that more fresh air is needed to keep the air at the breathing level reasonably pure. In an ordinary weaving-shed, ventilated through the roof, we found that the excess of carbonic acid rose to four times as much when the

gas was lit. There were $2\frac{1}{3}$ small (No. 4) gas-jets per person present. The details of this observation are given later.

The relative increase of air-vitiation in any given work-room after gas is lit depends also upon the proportion of gas-jets to persons present. This proportion was found to differ very greatly in different work-rooms. Where there is much machinery or floor-space to each worker the number of gas-jets may greatly exceed the number of workers. Thus in spinning-rooms there are often three or four gas-jets to each person: consequently the production of carbonic acid after gas is lit may rise to ten or twelve times what it was during day-light. On the other hand, in the more crowded rooms where sewing, &c. are carried on there may only be one jet to two or three persons, so that the production of carbonic acid is only about doubled after gas is lit, and the actual proportion of carbonic acid in the air at the breathing level may be scarcely at all increased if the vitiated air has free means of escape above.

Much may be done towards diminishing the vitiation of air through combustion of gas by avoiding wasteful methods of burning it. The following table (p. 428) shows the results of a series of experiments which I made on the light obtained for a given consumption of gas with various forms of ordinary burners in common use. London gas was used, averaging at the time about 16·5 candle-power—i.e. giving a light of 16·5 standard English candles when burnt at a rate of 5·0 cubic feet (measured at 60° F. and 30·0 inches barometric pressure) per hour through the standard "London Argand" burner prescribed by the Metropolitan Gas Referees. The standard light used in the experiments was the official ten-candle pentane lamp of the Metropolitan Gas Referees. The results with incandescent mantles at the end of the table are quoted from a Report published by the German Association of Gas and Water Engineers (*Journal of Gas-lighting*, April 16, 1901).

It will be seen from this table how greatly the amount of light obtained per cubic foot of gas burnt varies according to the method of consumption. The light was 48 times as great with the best as with the worst method. With ordinary burners the best result is evidently obtained from those with the larger sizes of opening, and with the gas issuing gently. Thus, to take an extreme instance, the light from a No. 0 burner at full pressure was increased nine times when a No. 7 (so-called "economiser") was slipped over it, so that the gas which passed at high velocity from the No. 0 burner underneath issued at low velocity from the much wider opening of the No. 7 burner above. The

Description of Burner	Pressure in inches of water between tap and burner	Consumption of gas in cubic feet per hour	Light in candles	Light in candles per cubic foot of gas burnt
Standard "London Argand"	—	4·86	16·0	3·29
"Union" or "fish-tail" No. 8	1·7*	12·6	22·6	1·79
	1·4	11·2	24·0	2·14
	0·8	8·2	23·7	2·87
	0·4	5·6	17·5	3·12
	0·2†	3·15	9·1	2·89
"Union" No. 6	1·8*	10·0	12·8	1·28
	1·2	8·1	15·7	1·94
	0·8	6·25	14·3	2·29
	0·4	4·15	10·0	2·41
"Union" No. 4	2·0	9·4	6·1	0·65
	1·7	8·3	8·9	1·67
	1·2	6·7	9·4	1·40
	0·8	5·1	8·4	1·65
	0·4	3·6	6·8	1·89
"Union" No. 2	1·8	5·5	3·45	0·63
	1·2	4·5	3·45	0·77
	0·8	3·8	3·5	0·92
	0·4	2·4	2·8	1·17
	0·2†	1·45	1·9	1·34
"Union" No. 0	1·9	4·5	1·6	0·36
	1·2	3·5	1·7	0·49
	0·8	2·7	1·6	0·59
	0·4	1·55	1·3	0·84
	0·2†	0·97	0·88	0·91
"Batswing economiser" No. 7, placed on "Union" No. 0	1·9	4·5	14·7	3·27
	1·2	3·5	11·0	2·97
	0·2†	0·97	2·1	2·16
"Union economiser" No. 6, placed on "Union" No. 2	2·3	5·9	13·9	2·36
	1·8	5·25	12·4	2·36
Common iron "Batswing," no number (irregular flame)	0·6*	16·0	37·3	2·33
	0·4	13·0	37·7	2·90
	0·3	10·5	34·2	3·26
	0·2	7·6	25·3	3·33
	0·15	5·5	17·8	3·24
"Batswing" No. 7	1·1	12·0	26·2	2·18
	0·7	9·5	22·9	2·41
	0·4	6·1	18·2	2·98
Cone-top burner, no number	1·8	6·8	21·6	3·18
	1·2	4·55	14·5	3·19
	0·5†	1·8	4·55	2·53
Cone-top governor burner	2·0	4·95	16·05	3·24
	1·0	4·8	15·65	3·26
Average of incandescent mantles	After 1 hour's use		—	—
	" 24 "	—	4·25	73·9
	" 100 "	—	4·25	70·3
	" 300 "	—	4·25	62·2
	" 600 "	—	4·25	56·4
		—	4·25	53·8
		—	4·25	53·8
		—	4·25	53·8
		—	4·25	53·8
		—	4·25	53·8

* Flaring.

† Small flame.

difficulty in securing a satisfactory result at all times with a given burner arises largely from the fact that the pressure in the main pipes is often allowed to fall very low. Where this is not the case a good result can be secured by using suitable burners and placing a pressure-governor on the supply pipe, or by using governed burners. In London there is a legal minimum (1 inch of water) to the pressure permitted in the main pipes.

The table shows clearly the great advantages of incandescent mantles. Their much more general employment in factories and workshops is very desirable, with a view to avoiding excessive vitiation of the air and at the same time obtaining a good and perfectly steady light.

By the use of the incandescent electric light all the inconveniences due to air-vitiation and heat from gas-jets can be avoided, though the extra expense as compared with incandescent gas-light is usually considerable. The arc electric light, so shaded that only reflected light falls on employees and machines, is sometimes used with great advantage.

The most wasteful methods of burning gas are still very commonly used in English factories and workshops, in spite of the greater expense and increased vitiation of the air; and there is much room for improvement in this respect.

Influence of Cubic Space per Person.

In the following table our observations are arranged so as to show the relations between the air-space per person and the proportions of carbonic acid in the air. Where, as was often the case, several analyses had been made of the air in one room the average for day-light or gas-light in that room has alone been counted in constructing the table, so that the general average may be as fair as possible.

Cubic feet per person	Under 300	300 to 400	400 to 600	600 to 1000	1000 to 1500	1500 to 2000	2000 to 5000	Over 5000
Average cubic feet per person	233	339	496	760	1227	1689	2906	9404
Volumes of CO ₂ { Day-light or electric light per 10,000 { Gas-light or lamp-light	11·4 20·1	10·6 13·6	9·7 14·0	10·2 13·8	9·2 17·4	9·0 19·0	7·1 17·8	12·8 26·3
No. of rooms { Day-light or electric light examined { Gas-light or lamp-light	36 14	33 8	28 15	27 18	27 14	25 9	24 5	25 12

It will be seen from the table that there was no general decrease in the carbonic acid with increase in the cubic space per person; and indeed the highest results were obtained, curiously enough, in the rooms with most space per person. This was evidently due partly to a large number of these rooms being spinning-rooms, which are commonly kept tightly closed in order to prevent cooling. In gas-lit rooms there is on the whole a marked relative increase in carbonic acid in rooms with a large cubic space per person. This is explained by the fact that in such rooms the proportion of gas-jets to persons is usually much greater than in rooms with a small cubic space per person. It is quite clear from the table that a large cubic space per person affords no guarantee for purity of the air. In factories and workshops, where rooms are always continuously occupied for some hours, foul air is about as often met with in sparsely occupied as in crowded rooms.

Influence of Time of Occupation.

In any occupied room a certain interval will elapse before the impurity of the air reaches an amount beyond which it does not further increase. The larger the air-space per person and the smaller the air-supply per person the longer will be this interval. In examining by chemical analysis the ventilation of a room it is frequently of importance to know whether the respiratory impurity of the air has already reached its probable maximum, or if not, how much higher the impurity is likely to increase.

In order to make calculations on these points it is first of all necessary to know the probable amount of carbonic acid given off per person and per hour in the room. In any particular person this amount varies considerably according to the amount of muscular work being done at the time. During great muscular work the amount may temporarily rise to ten times as much as during rest. The average for the 24 hours can best be calculated from the average daily consumption of food, which is pretty accurately known, and corresponds in the case of an adult man to an energy-value of about 3500 calories. Allowing for non-absorption of a small part of the food, and for the fact that the greater part of it consists of carbohydrate, the average production of carbonic acid for an adult man must be about 22 cubic feet per day or 0.9 cubic foot per hour. During complete rest only about 0.6 cubic foot per hour is given off, however: hence during the hours of activity about 1.1 cubic foot per hour is probably produced. A woman produces about

a fifth less than a man. In a factory about 1 cubic foot per hour may therefore be taken as a probable average quantity per person, though a higher estimate would be needed in cases where there is a good deal of muscular exertion.

The following table, for the calculation of which we are indebted to Mr P. J. Kirkby, Fellow of New College, Oxford, furnishes an easy means of estimating the probable maximum to which the proportion of carbonic acid in the air of a room will ultimately rise, and the rate of ventilation, assuming the latter to remain constant and the mixture of the air to be fairly complete.

$\frac{E}{E_0}$	$\frac{T}{t}$	$\frac{E}{E_0}$	$\frac{T}{t}$	$\frac{E}{E_0}$	$\frac{T}{t}$
·95	10	·72	1·43	·50	·62
·93	7·5	·69	1·25	·48	·59
·90	5	·66	1·1	·45	·53
·87	3·3	·63	1·0	·43	·50
·85	3·0	·61	·91	·40	·45
·82	2·5	·58	·83	·37	·40
·79	2·0	·56	·77	·35	·37
·77	1·8	·54	·71	·30	·31
·75	1·67	·52	·67	·25	·25

To use the table it is first necessary to calculate the excess of volumes of carbonic acid per 10,000 of air which would have been present with no ventilation at all. As each person produces about a cubic foot of carbonic acid per hour this number (E_0) is found by multiplying the persons present by the time in hours of occupation, and dividing the result by the cubic feet of air-space in the room divided by 10,000. Thus if the room has a capacity of 50,000 cubic feet, and 150 persons have been present for half-an-hour

$$E_0 \text{ will be } = \frac{150 \times 0\cdot5}{\frac{50,000}{10,000}} = 1\cdot5.$$

The ratio of the observed excess (E) to E_0 is then calculated. Thus if 9·5 volumes have been found in the air E may be taken as $9\cdot5 - 3\cdot5 = 6$, if the room is in a large town; and the ratio $\frac{E}{E_0}$ will be $\frac{6}{1\cdot5} = 0\cdot4$. The maximum to which E will subsequently rise is then found by multiplying E_0 by the number standing opposite to the value of $\frac{E}{E_0}$ in the second

column of the table. As in the supposed case this value is 0.4, and the number opposite is 0.45, the required maximum value of E is $15 \times 0.45 = 6.7$, so that the carbonic acid will ultimately rise to $6.7 + 3.5 = 10.2$ volumes per 10,000.

If the ratio $\frac{E}{E_0}$ is less than the least number in the first column, the corresponding number in the second column is the same, as the two columns have reached an equality. In this case the maximum proportion of carbonic has been reached, and there will be no further vitiation. Practically speaking, if the ratio $\frac{E}{E_0}$ is less than a third the maximum has been reached: if the ratio $\frac{E}{E_0}$ is greater than unity it is pretty certain that the air of the room was not pure to start with or that gas has been burning, or impure air entering the room.

The numbers in the second column of the table are in each case the ratio of the time (T) required for the entry of a volume of air sufficient to fill the room to the time (t) during which the room has been occupied. It is thus easy to calculate the value of T ; and the cubic capacity of the room divided by T gives the number of cubic feet of air per hour being introduced. Thus in the above example, since $\frac{T}{t}$ was 0.45, and t was 0.5, $T = 0.5 \times 0.45 = 0.225$, and the ventilation per hour was $\frac{50,000}{0.225} = 222,000$ cubic feet, or $\frac{220,000}{150} = 1480$ cubic feet per person.

It must always be borne in mind that the temperature of a room frequently increases up to a certain point with the duration of occupation, and that this may increase the rate of ventilation, so that the excess of carbonic acid will not actually rise so high as the calculated excess. The accuracy of the calculation is also limited by the fact that the production of carbonic acid per person may be somewhat greater or less than one cubic foot per hour, according to the nature of the work, &c.

For practical purposes the following abbreviated table will be found useful.

When the value of $\frac{E_0}{E}$ is	3 or more	2	1.75	1.5	1.25
The probable maximum future excess will be $E \times$	1	1.24	1.4	1.7	2.7

The probable number of cubic feet of fresh air per person and per hour is obtained by dividing 10,000 by the value obtained for the maximum future excess.

In taking samples of air we, so far as possible, selected times at which the impurity of the air would have reached its maximum. Hence it is possible to calculate the probable average air supply per person and the time required for the air of the room to be changed (*i.e.* for a volume of air equal to that of the room to enter) in the various rooms examined. The following table gives the average results for rooms of different sizes, the outside air being assumed to contain 3·5 volumes per 10,000 of CO₂.

Cubic feet per person	Under 300	300 to 400	400 to 600	600 to 1000	1000 to 1500	1500 to 2000	2000 to 5000	Over 5000
Average cubic feet per person	233	339	496	760	1227	1689	2906	9404
Volumes of CO ₂ per 10,000 { day-light or electric light }	11·4	10·6	9·7	10·2	9·2	9·0	7·1	12·8
Air supply per person in { cubic feet per hour }	1266	1408	1613	1493	1754	1818	2777	1075
Time required for air of room { to be changed, in hours }	0·18	0·24	0·31	0·51	0·70	0·93	1·05	8·7*

* Minimum figure.

The most striking fact brought out by this table is the rapidity with which the air was changed in the more crowded, as compared with the less crowded rooms. In the rooms with less than 1000 cubic feet of air-space per person the air was on an average changed in from 0·18 to 0·51 hours, so that as explained above, the air would reach its maximum impurity in from 0·54 to 1·53 hours. In the rooms (mostly spinning-rooms) with over 5000 cubic feet per person, on the other hand, the air-exchange was so slow that the maximum impurity could not have been reached during the hours of occupation, and the air could not have been pure on starting work again next morning. That this was actually the case we ascertained by special observations in one or two cases. Thus in two spinning-rooms with an average of 9840 cubic feet per person we found that after 4½ hours of occupation the average proportion of CO₂ was 10·6 volumes, and 5 hours later just before work stopped, 16·7 volumes. In two other similar spinning-rooms visited at 6·30 a.m. just as work commenced, the average proportion of CO₂ was 7·9 volumes. It was also found that in rooms of this

class when gas was burnt in the morning and evening the proportion of CO_2 remained very high all day. Thus in one room with 10,170 cubic feet of air-space per person 46.2 volumes were found about six hours after gas had been extinguished, whereas in the same room under similar conditions, but at a time of year when no gas was used, only 16.5 volumes were found at the end of the day's work.

Circumstances affecting Natural Ventilation.

Most of the rooms examined by us were ventilated by "natural" means—*i.e.* without the use of fans or other artificial methods of producing a current of air. In many of the rooms there were no special openings for ventilation. The rate of natural ventilation must evidently depend on a number of variable factors such as the amount of wind, the difference of temperature between inside and outside, the permeability of the walls and roof, the existence of various openings, &c. In all rooms a certain amount of exchange of air occurs through the walls, roof, floor, and various chinks, as was originally proved experimentally by Pettenkofer.

Since it was important to obtain some definite data as to the amount of air which under ordinary circumstances passes through various kinds of rooms unprovided with special means of ventilation, I made a number of special experiments on this point. The method usually adopted was to leave a certain number of paraffin candles burning at even intervals over the floor of the room. From the weight of paraffin burnt in a given time the volume of CO_2 produced (which was found by experiment to be .058 cubic foot at 15.5° and 760 mm. pressure per gramme of candle burnt) could be estimated, so that from the excess of carbonic acid in the room above that of the outside air the volume of air entering the room could easily be calculated from the table already given. The percentage of carbonic acid from the candles was in some experiments somewhat higher nearer the roof or on one side, but the calculations are based on the analyses of samples taken at the centre of the floor and at the breathing level. In the experiments on the first room the ventilating effects of an open fire-place are also shown. In all cases the rooms and surrounding rooms were thoroughly ventilated before starting, and since the buildings were practically in the country the proportion of carbonic acid could safely be assumed to be as nearly as possible 3.0 volumes per 10,000 in the room before starting and in the outside air. If any person was present during the experiment the carbonic acid

produced by him was allowed for. The analyses were by the method referred to above. The details of the experiments are stated in Appendix II. of the Committee's Report. The results are summarised in the following table (pp. 436 and 437).

These experiments, along with our observations in factories and workshops, throw a good deal of light on various circumstances which affect natural ventilation. Taking first the case of rooms with no fire-places or other openings, it will be seen that in the two small rooms (A and C) of 1100 and 1400 cubic feet, with boarded floors above and below, and no appreciable wind, the air was changed in from 2 to 3 hours, while in the larger room of the same character (D, 5600 cubic feet) the rate of change was once in 3 to 5 hours. It is evident that, the form and general construction being the same, the larger a room the more slowly will the air in it be changed by penetration of air through the walls, &c.: for the extent of wall, roof, and floor surface does not increase in the same proportion as the cubic capacity. The surface increases as the square, and the capacity as the cube, of any corresponding diameter, for rooms of the same shape. Thus an increase of 8 times in the capacity will correspond to an increase of only 4 times in the surface. Very large rooms, when unprovided with openings for ventilation, may thus contain foul air, although the air-space per person is very large. Many striking examples of this were met with in factories. Thus in a spinning-room of 91,500 cubic feet, containing only 9 persons as sources of vitiation, the carbonic acid during the day was found to rise as high as 16·5 volumes per 10,000, and this in spite of the fact that the temperature was extremely high (33°), which would naturally favour the exchange of air. The rate of change of air was apparently not more than about once in 24 hours. The apparently anomalous fact that we found the carbonic acid on the whole highest with a very large air-space per person, even with no gas burning, is to a great extent explained by the fact that the rooms with a very large air-space per person were relatively very large.

Structural differences, such as varying permeability of roof or extent of outside wall, may greatly affect natural ventilation. Thus in rooms B, F, G, and H, in which the construction favoured ventilation, the rate of ventilation with all openings closed, and no wind, was greater than would otherwise have been expected from their cubic capacity. The influence of an easily permeable roof was very clearly shown in some of our observations on weaving-sheds. In most weaving-sheds the roof is fairly permeable, so that in spite of the very large cubic capacity the

Description of Room	Duration of experiment in hours	Temperature		Hours required for a vol. of air equal to that of the room to enter	Remarks
		Inside	Outside		
Room A. Capacity 1390 cubic feet, 9·3 feet high, bedroom on first-floor, fire-place and one window, one outside wall of brick, walls and ceiling papered	11·6	17·2° to 17·8°	16·7° to 10·6°	2·1 to 3·4	Flap of fire-place closed. Breeze scarcely perceptible
Same room	12·5	17·9° to 18·3°	14·5° to 11·0°	1·3 to 1·8 0·6 to 0·7	Strong wind throughout experiment. Flap closed Flap open
Same room	14·0	18·3° to 17·4°	15·8° to 10·2°	0·9 to 1·3 1·7 to 2·4 0·7 to 0·8	Flap closed. Moderate breeze Breeze very slight Lower sash of window raised 7 inches
Same room	5·6	16°	14·2°	0·8 to 1·1	Flap open. Very slight breeze
Same room	3·7	16·7°	9·0°	0·9 to 1·0	Flap open. Very slight breeze
Same room	3·6	19·4° to 18·8°	13·5° to 13·8°	0·2 to 0·4	Fire burning in grate. Slight breeze
Room B. Attie of irregular shape, 786 cubic feet, one window, walls papered	3·2	17·8°	11·0° to 10·5°	1·2 to 1·8	Wind imperceptible
Room C. 1100 cubic feet, 11·5 feet high and nearly square, laboratory room on ground-floor, one window, one outside wall of sandstone, one inside wall of sandstone, and two of wood and plaster, walls not papered	9·2	13·0°	8·0°	1·5 to 1·6	Slight breeze
Same room	9·3	13·9°	4·5°	2·4	No breeze
Same room	8·0	13·5° to 13·6°	11·5° to 9·0°	1·3 to 1·5	Wind scarcely perceptible. A fixed ventilator opened at roof, opening about 24 sq. inches
Same room	4·0	17·2°	14·5°	0·25 to 0·30	Strong wind throughout experiment. Ventilator open

Description of Room	Duration of experiment in hours	Temperature		Hours required for a vol. of air equal to that of the room to enter	Remarks
		Inside	Outside		
Room D. 5600 cubic feet, 11·5 feet high, and nearly square, laboratory room on ground-floor, two double windows, one outside wall of sandstone, one inside wall of sandstone, one of brick and one of wood and plaster, no fire-place, no paper on walls	9·25	13·8° to 14·0°	11·8° to 9·2°	4·1 to 5·2	Wind scarcely perceptible. Simultaneous with third exp. on Room C
Same room	11·2	13·0°	5·0°	3·0 to 3·4	Slight breeze
Same room	3·8	17·8°	14·5°	1·4 to 1·9	Strong wind. Simultaneous with fourth exp. on Room C
Room E. 18,800 cubic feet, 11·5 feet high and 70 × 24 feet, a long laboratory room on ground-floor, 12 windows and 4 doors, 70% of wall is to outside and of sandstone, otherwise like room D	7·2	13·5°	11·8° to 9·2°	1·8 to 2·5	Wind scarcely perceptible. Simultaneous with first exp. on Room D
Same room	4·2	17·5°	14·5°	1·5 to 2·0	Strong wind. Simultaneous with third exp. on Room C
Room F. 13,300 cubic feet, 15 feet high and nearly square, ground-floor, one large open fire-place and two doors, two outside walls of stone	6·7	16·8° to 17·8°	15·6° to 14·0°	1·9 to 2·9	Gentle breeze. Chimney open and considerable draught up it. Simultaneous with first exp. on Room G
Room G. 75,000 cubic feet, 30 feet high in centre, stone walls and sloping ceiled roof with skylights, ventilators closed. Town-hall of Auchterarder	8·5	15·5° to 17·0°	14·4° to 12·2°	2·0 to 2·4	Gentle easterly breeze. Roof probably easily permeable to air
Same room	6·6	16·0° to 18·5°	16·7° to 17·3°	2·5 to 3·5	Wind scarcely perceptible
Room H. 72,000 cubic feet and 28 feet high in centre, side windows, ventilated by openings in roof measuring about 46 square feet in all, and communicating with loft below slates. Free Church, Auchterarder	5·5	11·7° to 16·0°	12·7° to 13·8°	2·2 to 3·3	Gentle breeze

exchange of air is usually considerable, even when all ventilators are closed. In some sheds, for instance, we found that with all, or nearly all, ventilators closed, and about 1500 to 2500 cubic feet per person, the carbonic acid during the day only rose to 6 or 7 volumes per 10,000. In one shed, however, the roof, instead of being of the usual saw-back construction, was covered with a sheet of water for coolness, with skylights projecting through. The roof was thus exceptionally airtight. Observations made in this shed at a time of year when all ventilators were kept closed gave the following results. The shed was about 12 feet high, with a capacity of 388,800 cubic feet, and 1620 cubic feet per person present.

Vols. of CO ₂ per 10,000		
10.40 a.m.	Centre of shed	24.4
10.45 "	N.W. corner	24.8
10.50 "	S.W. "	25.2
11.15 "	N.E. "	25.6
11.30 "	S.E. "	25.6
5.0 p.m.	N.E. "	30.8
5.10 "	S.W. "	31.2
5.15 "	S.E. "	33.0
5.30 "	Centre	33.0

These results show a rate of ventilation far lower than in any other weaving-shed which we examined.

The influence of an open chimney, with or without a fire burning in it, is very distinct in the experiments on room A. A bright fire increased the ventilation of room A as much as ten times, and the mere opening of the flap of the grate doubled the ventilation. The influence of opening a window in room A and of a small ventilator in room C is also shown.

The influence of wind in increasing natural ventilation is shown very distinctly. In rooms A, C, and D the ventilation was increased by from two to six times by a strong wind.

Difference of temperature between inside and outside must affect the exchange of air, particularly if the roof is easily permeable, or contains openings, and when the whole of a high building is heated. The effects of temperature were not, however, clearly apparent in the candle experiments; and it must be remembered that the driving pressure due to ordinary differences of temperature is slight as compared to that due to even a gentle breeze. In the following observations, made in a large and well-ventilated weaving-shed at

Auchterarder, the effects of temperature are clearly seen. The shed was of 472,000 cubic feet capacity, and about 16 feet high, with the usual saw-back roof. There were 72 10-inch cylindrical ventilating pipes in the roof, all open, and the air-space per person was 2,350 cubic feet. Work began at 9 a.m. During the dinner-hour (1 to 2 p.m.) about half the employees remained in the shed. There was an easterly breeze during the observations. An analysis of the outside air gave 2.8 volumes of CO₂.

	Temperature		Volumes of CO ₂ per 10,000
	Inside	Outside	
10.8 a.m. Centre of shed	12.2°	11.7°	5.3
10.40 " " "	13.8°	12.6°	6.2
11.40 " " "			7.3
12.20 p.m. " "	15.5°		{6.6
12.50 " " "	16.3°	15.3°	{6.8
1.40 " " "			6.8
2.0 " " "	18.2°		{4.6
3.55 " " "	22.6°	13.8°	{4.4
			{3.7
			{4.2
12.35 p.m. West side of shed	15.5°		7.9
1.47 " " "	16.3°		7.0
4.10 " " "	22.6°	13.8°	4.0
12.47 p.m. East side of shed	15.5°		5.0
1.55 " " "	16.3°		4.6
4.17 " " "	23.7°	13.8°	2.7

During the afternoon the inside temperature rose rapidly, in consequence, chiefly, of the sun shining in through the windows, which faced west. It will be seen that as the temperature rose the excess of carbonic acid in the air gradually fell to about a fourth of what it had been in the morning. An interesting point also shown by the analyses is that the air was much less vitiated on the east than on the west side of the shed. This was almost certainly due to the easterly breeze.

The observations in this shed were repeated in winter, when gas was being used in the evening, and all but nine of the ventilators were closed. There was 2560 cubic feet of air-space per person. The shed was heated by steam-pipes. The gas-jets (429 No. 4 Bray's burners) were lit between 3.45 and 4 p.m. The results were as follows.

				Temperature		Volumes of CO ₂ per 10,000
				Inside	Outside	
10.0	a.m.	Centre of shed				5.5
10.38	"	"	"	11.0°	3.5°	6.0
11.18	"	"	"	12.0°		6.0
2.20	p.m.	"	"	13.4°		4.8
3.35	"	"	"		5.5°	6.0
4.35	"	"	"			15.8
4.45	"	"	"			14.8
5.10	"	"	"			14.6
10.30	a.m.	East side of shed		11.0°	3.5°	5.9
11.30	"	"	"			6.2
3.30	p.m.	"	"	15°	5.5°	5.9
4.50	"	"	"			5.7
5.40	"	"	"	17.5°		16.5
						14.2
10.45	a.m.	West side of shed				5.4
11.35	"	"	"	12.7°		5.7
2.35	p.m.	"	"	14.2°		5.1
3.25	"	"	"		5.5°	5.1
5.0	"	"	"			6.0
5.45	"	"	"	17.5°		14.6
						14.8

It will be seen that in spite of nearly all the ventilators being closed the CO₂ did not rise so high during day-light as during the forenoon in the previous observations, when the temperature inside was nearly equal to that outside. On this occasion there was a very slight westerly breeze, and the CO₂ was lowest on the west side of the shed. With the gas lit the excess of CO₂ increased four times, there being $2\frac{1}{3}$ gas-jets to each person present. An analysis of the outside air gave 2.8 volumes of CO₂. The roof of the shed was evidently very permeable to air.

When, as frequently happens in factories, &c. two or more floors are in free communication by stairs, lifts, or other openings, the vitiated air will pass upwards. We observed this in a number of cases. Thus in a printing establishment the air was found to contain 10.1 volumes of CO₂ per 10,000 in the basement and 24.5 volumes in the second-floor. The air entering the second-floor by the shaft of the lift contained 16.5 volumes. In another building the air entering from below by the stairs contained 13.8 volumes, and in the room itself 18.8 volumes. In another case the air of an empty upper room, with no persons or lights in it, contained 11 volumes. In another case the air entering

from below by a grid contained 12·8 volumes, and the room itself 15·5 volumes. We noticed that as a general rule the air in basement or ground-floors is relatively pure. Basement and ground-floor rooms commonly act as intakes for whole buildings.

The openings for ventilation in factories and workshops are usually ordinary windows. For most rooms this seems to be the most practical arrangement; but the opening and closing of windows require constant attention, as their action is dependent on varying conditions of weather. In well-managed rooms a foreman or other person is responsible for having enough of windows open to keep the air fresh without causing inconvenience from cold or draughts, and for the proper regulation of the heating arrangements. The most suitable arrangement of windows varies greatly in different kinds of rooms. They should always, however, open at as high a point as possible, with a view both to avoidance of draughts, and to allowing the more ready escape of the heated air from lights and persons. Windows so arranged that the incoming air can be directed upwards are advantageous in winter, but should also be capable of being opened freely in summer. The free opening of windows in summer is an enormous advantage.

In very wide rooms, sheds, &c. special ventilators or shafts in the roof are often provided, and may be supplemented by Tobin tubes or other inlet openings. We frequently observed that these ventilators were either totally insufficient in size, or had been blocked up in cold or windy weather, and left in this condition. Often, too, the shafts are so obstructed by various contrivances as to be of very little use except in windy weather, when they are least needed, as natural ventilation through other channels is then at its maximum. Ventilators which are well designed with a view to avoidance of draughts in windy weather, or to utilisation of the effect of wind, are often quite insufficient to give the necessary quantity of air in still weather, so that unless windows are opened the ventilation may be very bad. Roughly speaking a greater velocity than about 200 feet per minute or 12,000 feet per hour up a ventilating shaft can seldom be counted on in still weather, even with free inlets to the room. Hence to give a ventilation of 2000 cubic feet per hour, about one square foot of free outlet shaft would be needed for every six persons, together with corresponding inlet provision, if the ventilation depended entirely upon the ventilators.

In large and at the same time crowded rooms it is very difficult to provide adequate ventilation at all times except by the use of fans;

but observations such as those just quoted on the Scotch weaving-shed show that excellent results can be attained without mechanical ventilation even in very large rooms if there is no crowding. The difficulty in ventilating crowded rooms, if fairly large, without fans is well illustrated by the notoriously bad ventilation of elementary schools. The average proportion of carbonic acid in elementary schools during the winter months without fan ventilation was found to be 18.6 volumes, with an average of 168 cubic feet of air-space per child, and 15,450 cubic feet per room¹.

Ventilation by Fans.

Ventilation by fans has the great advantages that (1) practically unlimited quantities of air can be supplied; (2) the supply is completely under control, so that it can always be relied on; (3) the incoming air can be warmed, moistened, or filtered from soot; (4) dust and fumes can be removed at or near the points where they are given off. These advantages are so great as compared with the cost involved that where engine-power or electricity is available mechanical ventilation is now very largely used in factories, even in rooms which are not crowded.

A fan may be placed in either an inlet or an outlet for air, the best arrangement for any particular case depending on circumstances. If it is necessary to warm, filter, or moisten the incoming air the fan should, as a rule, be in an inlet, so that no untreated air can enter the room. On the other hand if the incoming air has not to be treated, the most convenient position is usually in an outlet placed high up. The incoming air then enters through the walls, roof, and various openings. The incoming air currents should be so directed and subdivided as to secure proper distribution of air, and reduce draught to a minimum. In rooms of great superficial area several fans are needed to secure proper distribution; and often a combination of inlet and outlet fans is advantageous. Where a fan is used for the removal of dust, steam, or fumes, which are escaping into the air and cannot be dealt with at their point of origin, the fan should be placed so as to draw off the vitiated air as directly as possible, and particularly not to draw it across the room. We found that mistakes as to this point are not infrequent. Proper heating arrangements must, of course, be combined with fan ventilation, whether or not the incoming air is heated. A short *résumé*

¹ Carnelley, Haldane, and Anderson, *Philosophical Transactions*, 1887, B, p. 79.

of details with regard to the power required, the arrangement of ducts, &c. will be found in Appendix II. of the Committee's Report.

Owing to the relatively large cubic space per person in factories (see above), and the fact that persons who are actively employed are less sensitive to draughts, fan-ventilation is more easy to arrange for in factories than in public buildings, and usually it is not necessary to warm the incoming air. Even in very crowded rooms, provided that the heating arrangements in winter are adequate, extraction fans placed high up in the walls or roof often answer very satisfactorily if the inlet openings are suitably distributed. For instance in a fully occupied room in a chocolate factory, with 153 persons present and 380 cubic feet of air-space per person, the average proportion of CO_2 was found to be only 4.9 volumes per 10,000. In a very overcrowded ordnance workshop, with 200 persons present, 19 gas-jets lit, and only 155 cubic feet of space per person the average proportion was 8.2 volumes. In both these instances the ventilation was by extraction fans in the walls.

Standards of Purity.

By the Factory Act of 1901 the Secretary of State is empowered to prescribe a standard of "sufficient ventilation" for any class of factories and workshops. No definite standard of purity has, however, hitherto been legally fixed, except in the case of cotton-cloth factories, in which artificially humidified air is employed. For these factories the Factory Act provides that in no part of the factory shall the proportion of carbonic acid in the air be greater than 9 volumes per 10,000. In practice this standard is only enforced when no gas is burning. After careful consideration of the circumstances in factories and workshops the Committee has recommended that a general standard be prescribed to the effect that the ventilation be such that the proportion of carbonic acid at the breathing level shall not rise during day-light or with electric light beyond 12 volumes per 10,000, and during gas-light beyond 20 volumes: the only exception to be during fogs, or in factories where carbonic acid is produced in other ways than by respiration and combustion. Compliance with this legal standard would imply that under average conditions of weather &c., the proportion of carbonic acid should be usually under 10 volumes per 10,000. It would also imply that, assuming each person to produce about 1 cubic foot of carbonic acid per hour (see above), at least 1200 cubic feet of air per person and per hour should be supplied.

A much larger air supply is doubtless desirable, and is actually supplied in a large number of factories, but a more stringent *legal* standard would probably prove impracticable. One main cause of the bad ventilation which exists in many factories and workshops is apparently an objection to fresh air on the part of a small minority of the employees. Another not infrequent cause is insufficient warming where the work is sedentary. The laying down of the standard proposed would probably lead to the objections being overcome, and to much more attention being given to the proper utilisation of existing means of ventilation and warming. A carbonic acid standard would also supply a definite test of the degree of efficiency of any means of ventilation, and thus greatly encourage the provision of really good ventilation with a minimum of wasteful expenditure.

THE DIGESTIBILITY OF THE ALBUMINOUS CONSTITUENTS OF HUMAN MILK AND THAT OF VARIOUS SUBSTITUTES FOR IT.

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THE most important of the proteid constituents of milk is casein. From 70 to 90 % of the total proteids of milk consists of this substance¹. In milk, casein exists as a soluble calcium compound, which is not precipitated by boiling, but from which casein is deposited by the addition of acid or by the action of rennet². This phenomenon is known as the curdling of milk and is a preliminary to its gastric digestion, both the acid in the stomach and the rennet being capable of producing it. By artificially influencing the nature of its curd we can influence the digestibility of the milk. In human gastric digestion and in the experiments detailed below both acid and rennet are present. The curdling of milk is a necessary preliminary to its digestion in an acid medium, and by influencing this process we can also influence the digestibility of the milk.

In cow's milk the addition of a few drops of acid or rennet occasions in a few minutes the precipitation of a hard toughish coagulum, whereas if we treat human milk or one of the prepared milk foods, for instance Fairchild's Peptogenic Milk, in the same way, a more or less finely flocculent precipitate of casein is thrown down. In the case of human milk the floculi are very fine indeed. *Ceteris paribus* the fineness of the precipitate is an important fact in its digestibility.

¹ Our own figure is 93.5 %. Stutzer (*Milch als Kinder-Ernährung*, Bonn, 1895) gives without stating authority 40 %. Lehmann and Hempel (*Pflüger's Archiv*, vol. LVI. 1894, p. 577) 70 %.

² Hammarsten, *Zur Kenntniss des Kaseins u. der Wirkung des Lab-Fermentes*, *Abhandl. König. Gesellschaft zu Upsala*, 1877. Arthus and Pagés, *Archives de Phys.* 1890, p. 331.

This is partly to be explained mechanically, a finely divided casein offering, per unit weight, more surface for the action of the digestive juices than a casein deposited in lumps. Lumps further act as a mechanical irritant to the stomach and intestines, stimulating peristalsis. The lumps thus become hurried down the intestinal tract and are voided undigested.

We can influence the degree of fineness in which casein is precipitated in various ways. One of these is to dilute the milk and thus expose to the digestive juices a more dilute solution of casein. Another method is to diminish the amount of soluble calcium salts¹ present in the milk by the addition of *e.g.* an oxalate or citrate. A further method² consists in altering the reaction of the milk to be curdled and the strength of the acid used to curdle it. The stronger the acid the more tough and compact the curd and hence the greater the trouble to digest it. This fact is of interest in connection with the difficulty often experienced in digesting milk by patients suffering from hyperchlorhydria, which can often be entirely removed by the addition of an alkali to the milk.

The factor however of greatest importance in determining the digestibility of a casein is the nature of the casein itself. The casein obtained from human milk is chemically different to that obtained from the milk of any other animal. Wroblewsky³, working under Drechsel's guidance, found the percentage compositions of cow's and human casein to differ especially with regard to their sulphur and phosphorus contents. As the result of peptic digestion experiments these authors found that in the case of human milk no insoluble nuclein compound was split off; on the other hand in the case of cow's casein an insoluble nuclein residue invariably remained even after indefinitely prolonged peptic digestion. From these experiments Wroblewsky concludes that human and cow's casein are chemically different substances. According to Siegfried⁴ all the phosphoric acid in human milk is bound directly to "albumen," whereas in cow's milk only half of it is so bound.

The following experiments were undertaken to ascertain to what extent this chemical difference between human and cow's casein affects their respective digestibilities and also to what extent their differences

¹ Arthus and Pagés, *loc. cit.*

² Courant, *Ueber die Reaktion der Kuh- u. Frauen-Milch.* Inaug. Dissertation, Bonn, 1891, p. 39.

³ *Beiträge zur Kenntniss des Frauens Caseins.* Inaug. Dissertation, Bern, 1894.

⁴ *Zeitschr. für physiol. Chemie*, vol. xxii. p. 575.

in this respect can be compensated for by artificial means as adopted in various proprietary milk foods.

Method.

Since the best method at our command was digestion *in vitro* we were anxious to imitate physiological conditions as far as possible and to contrive so that the results were comparable *inter se*.

The cow's milk was obtained from a good London dairy. In submitting the artificial milk foods to digestion the directions on the label were followed exactly. The human milk, of which we obtained two litres, was kindly collected for us by one of the externe nurses of Queen Charlotte's Lying-in Hospital, London. It was derived from twenty-nine women in periods of lactation varying from six weeks to five months. The different portions of milk were well mixed before submitting the whole to analysis and digestion. The following table (Table I.) gives the composition of the milks as used for the experiments, with the exception of the cow's milk which was diluted with an equal quantity of New River tap water.

Three classes of digestive experiments were made: (1) Peptic, (2) Pancreatic, (3) Peptic and subsequently Pancreatic.

Peptic digestion. The average amount of milk in an infant meal is from 60 to 90 c.c., which in the case of human milk would represent about 1 gramme of total albuminoids. By preliminary experiments we satisfied ourselves of the efficiency of Fairchild's Pepsin and of the approximate accuracy of the data upon the label. We used therefore for our peptic experiments a digestive solution of the following composition. Pepsin (Fairchild's) 0·06 gramme, hydrochloric acid (33 %) 6·00 c.c., water 600 c.c. Of this solution 6 c.c. were added to 50 c.c. of the milk under experiment. The normal infant stomach is usually empty from one to two hours after the ingestion of a meal¹; but as a somewhat fine index was required the time for peptic digestions in these experiments was fixed at one hour. The temperature at which they were made was 38° C. At the end of the hour the mixtures were brought rapidly to the boil. The amount of albuminoids digested in each case was ascertained as follows. After the boiling the contents of each flask was washed on to a prepared filter which was provided with an ice and salt jacket. Filtration was allowed to continue until complete.

¹ Epstein, von Puteren, Wohlmann and others, quoted by Soltau Fenwick, *Disorders of Digestion in Infancy*, London, 1897.

TABLE I.

Showing the percentage composition of the milks etc. used for the experiments.

	Specific gravity	Water	Total solids	Ash	Total nitrog. matter	Total albuminoids	Casein	Fat	Milk sugar	Mal-tose	Dex-trine	Sol. starch	Starch	Cane sugar
Human milk	1.032	88.42	11.58	0.19	1.18	1.06	0.99	2.55	6.28					
Cow's milk	1.032	87.67	12.33	0.74	3.07	2.74	2.56	3.15	4.76					
Patent milk I	1.036	85.65	14.53	0.42	1.86	1.64	1.32	4.00	8.22					
" II	1.022	92.41	7.59	0.38	1.84	1.70	1.48	1.37	3.15	0.32	0.57			
" III	1.019	94.49	5.51	0.10	0.85	0.80		0.33	0.39			0.58	0.90	1.46
" IV	1.030	87.99	12.01	0.30	1.38	1.23	1.18	3.65	6.71					
" IV (a)	1.030	87.79	12.21	0.31	1.53	1.45	1.37	3.65	7.18					

The temperature during the whole time of filtration never exceeded 3° C. This was deemed important since filtration proceeded very slowly at ordinary temperatures. Owing to bacterial activity further decomposition of the respective mixtures might easily have taken place. When filtration was complete the residue was washed with a little water to free it more thoroughly from soluble albuminoids, and was subsequently removed to a Kjeldahl flask and the nitrogen estimated in the usual manner. The figure thus obtained was calculated to proteids and the result subtracted from the original insoluble proteid content. The remainder gave the quantity of proteid digested. The results of the peptic digestion are given in the respective tables.

Pancreatic digestion. For the pancreatic digestion a standard zymine solution was made up on the lines of that used by Biffi¹, with the exception that instead of powdered pancreas, Fairchild's zymine powder was used. This solution had the following composition: zymine powder (Fairchild's) 1·0 gramme, chloroform water 100 c.c., saturated sodium carbonate solution 1·0 c.c. This solution was allowed to stand for 12 hours at 40° C. and then filtered. In the pancreatic experiments 50 c.c. of the milks or feeding-bottle mixtures were taken; the time allowed was three hours; the temperature 38° C.; 10 c.c. of the above solution was added to each. During the whole time of digestion the reaction was kept alkaline. At the end of three hours the nitrogenous substances undigested were precipitated by potash alum according to Schlossmann's method, and nitrogen estimations made as above. The amount thus obtained calculated as proteid was then subtracted from the total albuminoids originally present, the remainder representing the quantity of albuminoids digested².

Peptic and Pancreatic digestions. In the living infant the milk ingested is submitted first to the action of the gastric juice, and the residue is subsequently exposed to the pancreatic juice. An attempt was made to imitate this *in vitro*. Quantities of milk (50 c.c.) were submitted to the action of the artificial peptic solution for 1 hour, were then filtered quickly at a low temperature (see above), the residue rinsed into a flask with 50 c.c. of water and made alkaline. The ensuing

¹ *Virchow's Archiv*, 1898, Band CLII. Heft 1.

² In some earlier experiments we attempted to estimate the albuminoids digested by the artificial pancreatic solution in a manner similar to that adopted in the case of the peptic digestion. The great difficulty of filtering the mixtures after pancreatic digestion, especially in the case of human milk and those milk preparations approaching it in digestibility, led us however to adopt the above method.

mixture was then submitted to the action of the pancreatic solution for three hours and subsequently treated as described under pancreatic digestion.

The results of these experiments are given below in tabular form. Each result is the mean of three or four experiments, the extremes never differing by more than 0·2 %. In the case of the last patent food we made two separate series of experiments with two samples obtained at different times, on account of the great difference in the results obtained with this and the other patent foods.

Methods.

All nitrogen estimations were made by Gunning's modification of Kjeldahl's method. The general factor for the calculation of proteids was 6·37, except in the case of human milk when it was 6·34 (Munk, *Virchow's Archiv*, vol. CXXXIV. p. 501). Total albuminoids were estimated by a slightly modified Ritthausen's method (*Journ. für prakt. Chemie*, N. F. vol. xv. 1877, p. 329). Casein was estimated by Schlossmann's method.

TABLE II.

Showing the digestibilities in vitro of the proteids of human and cow's milk, and those of certain artificial milk foods.

Name of milk	Digested after 1 hour's peptic digestion	Digested after 3 hours' pancreatic digestion	Digested after 1 hour's peptic and 3 hours' pancreatic digestion	Remarks
I. Human milk 6th week to 5th mo.	48·12 %	21·07 %	75·46 %	
II. Cow's milk 50 %	47·44 %	27·00 %	47·44 %	
III. Patent milk I	54·88 %	40·24 %	54·88 %	
IV. Patent milk II	55·29 %	24·11 %	56·47 %	
V. Patent milk III	26·25 %	32·50 %	40·00 %	This food contained no milk; it was deficient chemically, & contained starch & cane sugar
VI. Patent milk IV				
Sample A	28·46 %	32·52 %	35·96 %	} This milk was the dearest of all those examined and bore the name—"human milk"
Sample B	28·96 %	33·10 %	36·19 %	

CONCLUSIONS.

The above tables require but little comment. Physiologically the results confirm those of Wroblewsky and Siegfried showing that there is an essential difference between human and cow's casein, and especially that this difference affects the nucleo-proteid moiety of the casein molecule, or that part of it which is digested by the pancreatic in distinction to the part digested by the gastric juice. From the point of view of experimental dietetics the results show the importance, in estimating the digestibility of proteid substances *in vitro*, of submitting the residue of gastric digestion to the artificial pancreatic juice. It will be obvious from the above tables that in so far as concerns the simple gastric or simple pancreatic digestion, the digestibility of the proteid constituents of certain milk foods and indeed simple cow's milk itself closely approximates to or even exceeds that of human casein. When however we regard the total digestibility after peptic and pancreatic digestion we see that the substitutes for human milk fall considerably short of human milk itself. It will be obvious also from the table that certain milk foods do dietetically possess a considerable advantage over simple, unmanipulated cow's milk. From the point of view of public health the above results accentuate the nutritive advantage to the infant of mother's milk as opposed to any substitute for the same, in other words of breast-feeding as opposed to bottle-feeding.

A REVIEW OF CURRENT THEORIES REGARDING IMMUNITY.

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Concluded from page 285.

Two questions. In concluding our review of this division of the subject, which deals with the parts played by the bodily presence of cellular protoplasm as contrasted with the activity of the chemical products of that protoplasm, which from their solubility may act at a distance from the cell producing them, there are two points which may be alluded to. Firstly, it is difficult to see how, granted that two substances are necessary for bactericidal action, these substances can in the first instance at least meet the bacterium except within a cell. Unless all immune bodies are represented in the serum by go-betweens (*Zwischenkörper*),—by bodies of an identical nature and differing only in that they are subservient to the normal metabolism of the body—how, when a bacterium gains entrance to the blood, does it come in contact with the immune body for which its protoplasm has the affinity? The receptor, which fixes the group in the bacterium corresponding with it, is within a cell,—how can the affinities be satisfied until the bacterium gets within the cell? It is not till the cell has been robbed of the use of these receptors and they become over-regenerated that the immune body becomes free and can be found in the serum. It is possible that all immune bodies are represented by go-betweens, and that a certain amount of the latter must be kept free in the serum for ordinary metabolism, and it is also possible that when the gradient—to use a physical phrase—between the cell and the blood plasma becomes great so far as the amount of go-betweens present in the latter is concerned that the cell becomes more active and secretes more

of the material which is being more rapidly taken up than is usual in ordinary metabolism. Thus while the extra-cellular destruction of bacteria is easy to understand in the later steps of immunisation there is a difficulty about the earlier stages.

Secondly, if it is the case that in order to their destruction bacteria must meet in a cell immune body and complement produced by the same cell, why is it that during the process of immunisation only one of these cytases, to use Metchnikoff's phrase, is produced in excess? Why should the cell manifest so much activity in overproducing one of its products and manifest so little activity in producing another and an equally necessary substance?

The possible relationship of the processes occurring in immunity against bacterial action to processes normally occurring in the body.

In our review of the factors concerned in this variety of immunity we have seen that there is evidence of the vital activity of cells giving rise to the presence in the circulating fluids of the body of materials concerned in the death of bacteria. The question now to be looked at is whether the functions brought into play in the production of the protective bodies are called into existence only for protection against bacteria, and other noxious agents of a similar kind, or whether these functions are concerned in the normal activities of the cells. Two lines of enquiry might throw light on this matter, firstly, that concerned with asking the normal functions of the cells producing protective bodies; and, secondly, that which is directed to investigating whether or not bodies similar or identical with protective bodies occur in the sera of animals not the subjects of infection. To look first of all at this matter from the point of view of the cells. Here it is evident that the question arises of the normal functions of all the cells we have enumerated as concerned in the reactive processes already studied. Of these, we have seen reason to believe, the most important are the leucocytes, and we may direct our attention chiefly to them. What are the normal functions of these cells? Of this subject very little is known, and in fact it is probably by the study of immunity that information will be gained as to what under ordinary circumstances these cells do. From what indications we have we may say that they may have one or other of two functions. Firstly, their sole use may be to guard the body against infection. Secondly, such a function may be subsidiary to other functions. With regard to the

former of these possibilities we have to remark that in his early work on inflammation Metchnikoff⁽¹⁰²⁾ looked upon the leucocytosis which occurs in this process as its essential feature. This deduction was largely based on the phagocytic activities of wandering cells in the different types of the whole animal series from the lowest invertebrates upwards. In the lower animals such a phagocytic function of cells is simply the same process by which the animal seizes particulate bodies from the digestion of which it derives its food, and in many such animals these cells are the main, and it may be sometimes the only means by which food is obtained. The occurrence of phagocytic cells in practically all animals may be granted, though the homologies of the cells concerned in different species still require much investigation. Even, however, if the leucocytes of the mammalia are the homologues of the wandering cells of lower forms it does not follow that the functions of digestion and protection are parts of the same vital activities. As digestion in the higher forms has become chiefly a function of certain specialised tissues derived in the main from the hypoblast, it is possible that that of protection is the specialised function of the leucocytes; and while the latter do undoubtedly contain proteolytic bodies it is, as has been already pointed out, an assumption that these are identical with the bactericidal substances. There is little doubt that the body is more or less constantly exposed to the absorption of bacteria from the intestinal tract, though the extent of this risk requires further investigation, and there is little doubt that infection does not follow every such absorption, but the constant presence of bacteria in the neighbourhood of the intestinal lumen would not account for the marked leucocytosis which in mammals occurs after a meal is partaken of. It is to be remembered, however, that leucocytes can be attracted by such non-particulate substances as bacterial proteines, albumoses, etc., and it is a possibility that harmful materials of a similar nature require constantly to be disposed of in order that the body may not suffer injury. On the other hand it is quite possible that the occurrence of an increase of leucocytes in the circulating blood during digestion may indicate a digestive function on the part of these cells, though we have no facts by which we may judge what changes they may originate in the fluids being absorbed from the intestine. The general view of physiology is that the albuminous constituents of the blood are formed from peptone in the intestinal wall, *i.e.* during absorption, though as we shall see presently it is doubtful if the methods of physiological chemistry are sufficient to appreciate very fine differences which may exist between

albumins. There is no doubt there is thus an opening for further elaboration taking place in the fluid elements of the blood after absorption, and in these processes the white cells might play a part. If, as may thus be gathered, the functions of the leucocytes are obscure it is evident that it is still more difficult to form a conception of the parts ordinarily played in the body by the great variety of phagocytic cells to which we have alluded. In connection with this aspect of the subject there are numerous lines of enquiry opened out which would well repay following up.

Enquiries into the normal functions of the leucocytes being thus to a large extent barren, we proceed to ask if under normal circumstances substances analogous to protective bodies occur in the body. Here is involved the question of the co-relation of the bactericidal actions of normal sera with those of the sera of immunised animals, and regarding this the chief results of research have already been given. We have seen that complementary substances occur in ordinary sera which have a bactericidal action just as they form a very important part of the sera of immune animals, but we have seen that it is a matter of dispute whether they occur in the circulating blood. With regard to the free occurrence of immune bodies the case is different. We have adduced one instance where there occurs in a normal serum a body corresponding in all its properties with an immune body, and we have raised the question whether the bactericidal action of normal sera may not be always due to the presence of such bodies in addition to complements. The instance given was that of the body in the normal serum of the goat, which with the aid of complement from the horse will haemolyse rabbit's corpuscles. A very great number of such bodies having the properties of immune bodies have been discovered in normal sera, and the question arises what their normal function is, for it is, of course, inconceivable that, say, in the case given, the substance should exist in the goat's body for the purpose of haemolysing rabbit's corpuscles, when the latter happened to get into the goat's body. Just as in looking at this question from the point of view of the cells producing complement and immune body so here there are two main possibilities,—either the substances are concerned in the protection of the animal against infection or they are concerned in the elaboration of materials needed in ordinary metabolism. It is to the last view that Ehrlich inclines. A point which may be cited in its favour is derived from the analogies of what we have seen as probably happening in the case of tetanus toxine. The toxine molecules are, as has been observed, very

probably allied in nature to those which form the normal food of the cells fixing them. Here there is no question of the cellular receptors having a protective action, for it is because of these receptors being present in the cell that the latter becomes susceptible to the toxic action of the toxophorous group of the toxine.

But the probability that the substances we are concerned with are of use in ordinary metabolism is indicated by the fact that when materials analogous to the ordinary food materials of the body, and so far as we know incapable of harmful action, are introduced into the blood they are acted on by substances precisely similar to those which act on such a harmful agent as a bacterium. It was noticed by Bordet⁽¹⁰³⁾ that when rabbits were treated by intra-peritoneal injections of fowl's blood not only was a haemolytic serum obtained, but the serum when added to fowl's blood produced a precipitate somewhat like a coagulum. Subsequent investigations of similar conditions have shown that the reaction is one produced by the presence of the serum, and especially by the globulins of the blood injected. Of the results of such enquiries those of Myers⁽¹⁰⁴⁾ may be taken as an example. This observer found that intra-peritoneal injections, extending over two months, of crystallised egg-albumin into the rabbit's peritoneum produced a serum which caused a precipitate when added to solution of hen's egg-albumin. This precipitate was soluble in 2 per cent. sodium chloride solution and gave ordinary proteid reactions. The solubility of the precipitate thus marked the phenomenon off from a true coagulation. The serum had a slight effect on duck egg-albumin, but none on sheep globulin, sheep serum albumin, bullock serum, or Witte's peptone. The intra-peritoneal injection of serum globulin from the sheep into the rabbit gave rise to a precipitin (as the anti-bodies in this class of sera are called) capable of precipitating the globulin which originated it, but which had no reaction on bullock globulin or Witte's peptone. The injection of bullock globulin gave a precipitin which besides its specific action on the causal globulin also slightly precipitated sheep's globulin. The injection of Witte's peptone gave a precipitate with peptone, which precipitate, however, did not give the biuret reaction though soluble in 2 per cent. salt solution. The first three precipitins were not weakened by half-an-hour's heating at 56° C., and thus differed from immune sera, but in the case of the last precipitin a weakening occurred the effect of which, however, could be neutralised by the addition of serum from the normal rabbit. This precipitin therefore corresponded exactly with an immune serum. The important points regarding these

precipitins are, firstly, that bodies closely allied to the normal constituents of an animal's blood can originate in that animal's body substances capable of producing an effect upon themselves; secondly, that the anti-bodies thus produced in some cases resemble in their nature the anti-bodies of the serum of an animal immunised against bacterial infection, though in many cases this is not true; thirdly, that these anti-bodies are not so specific in their reactions as is the case with immune sera but are capable often of having a definite effect on substances allied to those which stimulated their origin. It is evident that by the discovery of these precipitins the way is opened up for believing that the go-betweens ("Zwischenkörper") often present in serum may have as a normal function the elaboration for the use of the body of the food materials after their preliminary digestion in the intestinal tract. How the precipitins originate has still to be investigated, and it is not known whether they are the products of leucocytic activity or what cells are concerned in their genesis, nor are their effects at all understood, for the nature of the precipitation which they cause is unknown. In fact it is doubtful if their effect *in vitro* is the same as their action when introduced into the body of an animal which contains in its blood materials on which they are capable of acting. Bashford⁽¹⁰⁵⁾ has pointed out that in a rabbit with a serum capable of precipitating peptone the injection of the latter substance into the ear-vein does not produce embolism which the injection of a precipitate caused by the same serum *in vitro* does. A final point may be noticed with regard to these bodies. The facts known indicate that in bodies so apparently identical when considered from the standpoint of ordinary physiological chemistry as serum globulin from the ox and that from the sheep, differences in constitution really exist which can be distinguished by this more delicate method of analysis by physiological reaction.

These facts regarding the appearance of substances in normal sera allied in nature to those found in immune sera, and the development of analogous substances by the injection of materials closely resembling the substances which must occur in the blood after a meal, open up the way for believing that in all probability the events of immunisation are closely related to what occurs under normal conditions in the elaboration of the many bodies required for cellular nutrition. Such nutrition may depend in many ways on the inter-action of affinities in food materials for receptors in the cells, and, from what we already know, we can realise that the process may be extremely complicated in nature. This

has been made clear by certain observations of Ehrlich and others. Not only can there be developed in the course of immunisation immune bodies and complements, but it is possible in many cases to get substances which can antagonise the actions of these. Thus if a serum containing complement be injected into the body of another animal there appears in the serum of the latter an anticomplement, *i.e.* a substance which when mixed with the latter and introduced along with an appropriate immune body can prevent the complement from acting with the immune body, and thus prevent the latter having any action whatever. Examples of the development of an anti-immune body are more rare, but Ehrlich has found evidence of their occurrence in the case of the immune bodies concerned in haemolytic action though they are apparently less common, if they can be developed at all, in connection with the immune bodies concerned in anti-bacterial action. In connection with this subject a very interesting and important matter arises. If an animal is treated with the corpuscles of another animal of the same species it will develop a serum haemolytic to the blood corpuscles of the second animal. It might be thought that this serum would be haemolytic to its own corpuscles, but this is never the case, although such a serum is frequently haemolytic for the corpuscles of other individuals of the same species. The following experiments illustrate this principle. Goat A was injected with the blood of three other goats, 1, 2, 3, and a haemolytic serum was obtained which acted on the corpuscles of goats 1, 2, 4, 5, 6, 9; it also acted slightly on the bloods of goats 3 and 8, and not at all on the blood of goat 7. Its own blood was also unaffected by the serum. Thus while there was evidence of an isolysin, *i.e.* a serum dissolving the blood corpuscles of animals of the same species, there was no evidence of the presence of an autolysin, *i.e.* the presence of a substance dissolving the animal's own blood corpuscles. Ehrlich has never obtained any evidence of the existence of such autolysins. Now if an ordinary haemolysin such as for example exists normally in the serum of the dog or the goat is injected into another animal there is produced an anti-haemolysin. Can such antibodies to the isolysin of the goat be produced? The serum of goat A was injected into a goat 10, and it was found that an anti-isolysin was produced, that is to say a body which protected some of the bloods mentioned as being susceptible to serum A against it. At the same time as goat A another goat B had been injected with the same bloods and it was found that at first for 14 days its serum showed the presence of very little isolysin. Afterwards an isolysin appeared in it, but it was

found to differ from that present in the serum of goat A in that bloods which were susceptible to A were not very susceptible to B. It was also found that the anti-isolysin of A had not the power to protect bloods against the isolysin B. Another goat C was treated in the same way as the two others, and again an isolysin was obtained which again showed different properties from A and B. Further, all these haemolysins A, B, and C, dissolved sheep's blood, so that in the latter there must have been three groups, one to take up the receptor of each of the three sera. The theoretical considerations involved in these three cases are very complicated, and Ehrlich states them as follows. Suppose that in a given red blood corpuscle there exists a group α and in the body of the animal into which it may be injected for the purposes of immunisation (in the case under consideration let the latter be one of the same species) there is an affinity which satisfies it which we shall call receptor α . Now under ordinary circumstances the latter will first be saturated and then reproduced in excess and cast off into the serum as immune body. But suppose that not only does group α exist in the red blood cell of the blood introduced for immunisation, but also in the blood cells or somewhere in the body of the host which is undergoing the immunisation process. If now receptors α are cast off in great numbers they may combine with the α groups, and if the latter are in the red blood cells, the fact that there is complement present in the serum may lead to haemolysis; this would be a case of the occurrence of an autolysin. But if the α receptors were only cast off at first in small numbers, and (as we have seen in the case of tetanus antitoxine) this may quite well be the case, then they would unite in small numbers with the α groups, would rob the cells containing them of material required in normal metabolism, would stimulate them in turn to be over-reproduced and to be shed off into the serum. The latter would then contain an anti-autolysin. Hitherto no evidence of the formation of such bodies as the latter has been obtained.

Evidence has, however, been brought forward of the possibility of the development of auto-anticomplements. Ehrlich and Morgenroth made the following observations. The normal serum of the rabbit possesses a complement and also a go-between which acting together can dissolve guinea-pig corpuscles, and further there is in the rabbit's blood also a complement which can activate the immune body present in rabbits treated with ox blood. It was found however, that if rabbits had been a week previously injected with goat serum in-

activated by heat these capacities were lost, and it was shown that the serum of these rabbits, especially when heated to 56° C., could prevent the complement normally present in rabbit's serum from acting. An anti-complement in other words had been developed, and, taking into account the fact that there was no complement present in the goat's serum, the formation of the anti-complement must have been due to a substance having a receptor identical with one already existing in the rabbit's body (*i.e.* in the normal complement of the rabbit). The anti-complement must thus have been of the nature of an auto-anticomplement. Such is the only exception that has as yet been found to the fact that the body seldom, if ever, produces antibodies to receptors already existing within it. It is the expression of what Ehrlich calls a "horror autotoxicus," on the part of the animal body. It is evident that these facts have a most important bearing on the co-relation of the processes of immunity with the enormously complex processes of normal metabolism. We may now therefore give a brief indication of their applicability in this direction.

From the consideration of the facts regarding the fixation of toxines Ehrlich⁽¹⁰⁶⁾ first arrived at the conception that unlike such materials as strychnine, etc., which show no tendency to become fixed to the cellular protoplasm the bacterial toxines have the capacity of being assimilated. Thus they proclaim their affinity to the food-stuffs of the cell which manifest the same property. This conception found support in the facts which have been detailed with regard to the effects produced in the body by the injection of serum globulin, peptone, etc. His view is that there exists in the protoplasm of the active cell a nucleus of vital activity with which the special capacities of the cell are associated. To this vital nucleus there are attached as side-chains atoms or atom-complexes which play an essential part in the work performed by the cell but which are not necessary to its life. Among these side-chains are the unsaturated affinities which normally fix food materials to the cell and which may fix such materials as toxines, the protoplasm of bacterial cells, etc. These fixative affinities are of very varied character, being adapted to the varying requirements of the cell. First of all there is the simplest form, which consists of a simple affinity and which is concerned in the fixing to the cell of relatively simple materials such as ferments, toxines and other cellular secretions. These simple affinities Ehrlich calls receptors of the first order. Such receptors when cast off in excess into the serum form such an antitoxine as that of diphtheria. If the molecules to be absorbed into the cell

are of large size then more complicated receptors are necessary. For making such a molecule fit for absorption it may be necessary that a preliminary digestive process should take place. Here the fixing receptor is supposed to fix the molecule by one affinity while another arm carries a ferment-like capacity by which the fixed molecule is broken up. These are Ehrlich's receptors of the second order. The example which he gives of such a receptor is the class of bodies concerned in agglutination. These substances can be heated to 70°C . before they lose their effect, and the addition of normal serum of the animal from which the agglutinine was derived has no effect in causing the agglutinating properties to return. The principal group of receptors, however, are those of the third order, which besides being attached to the cell contain two haptophorous groups, one of which fixes the food particle while the other fixes the ferment-like body (complement), the action of which is necessary for the breaking up of the particle fixed. These receptors include by far the greater number of the bodies we have been studying in connection with immunity against infection. When they are cast off into the blood by the same mechanism as in the case of antitoxine production, they form if they are normal factors in the latter the go-betweens, whereas they constitute the immune body if they are freed by a process of immunisation. To include both of these varieties, differing only in the stimulus calling them into existence, Ehrlich uses the term "amboceptors." To the whole orders of receptors, from the fact of their containing haptophorous groups, he gives the name of "haptines," it is by them that a cell is able to attach its food to its centre of vital activity. According to his view there exist in the bodily cells innumerable such affinities concerned in the preparation of food for cells and in elaborating substances for the use of other cells. Their multiplicity may be judged of by the numerous examples of free receptors already known, lysins, agglutinins, precipitins, complements, ferments, antitoxins, anticomplements, anti-ferments, etc. The multiplicity of closely allied haptines may be judged of by the specificity of the numerous haemolysins for their corresponding bloods, and even when only one blood is under consideration the existence of a number of immune bodies concerned in the same reaction may be judged of by the facts given regarding the occurrence of almost identical immune bodies in connection with the isolysin experiments.

The consideration of the theories advanced at the present day to account for the phenomena of immunity thus leads on to results which

have a deep biological significance. If looked at from the chemical standpoint alone they form a contribution, it may be said to be the as yet most far-reaching contribution, to our understanding of the complex processes by which living matter manifests itself as active. Not only so, but they lead to the possibility of understanding functions of cells, the laws which govern their activity, and the ways in which groups of cells in the complicated household of the animal body contribute to each other. Thus not only the pathologist but the biologist and physiologist are concerned in the solution of the problems which are opened up.

ERRATA.

- p. 218, line 8 from top:—"an animal immunised against the filtered toxines of the cholera vibrio was not immune against an injection of the living organisms and further that the serum of one animal immunised against the latter did not protect another animal against a fatal dose of the filtered toxine" should read "the serum of an animal immunised against the cholera vibrio did not protect another animal against intestinal infection with the cholera vibrio, *i.e.* was incapable of neutralizing the toxines produced by the latter which constitute the active pathogenic agent in such an infection."
- p. 243, line 16 from top:—after "Bordet" read "working with guinea-pigs treated by intraperitoneal injections of rabbit's blood and,"
- p. 243, footnote:—for "*sensibilatrice*" read "*sensibilisatrice*."
- p. 253, second line from bottom:—for "2 c.c." read "twice the simple dissolving dose."

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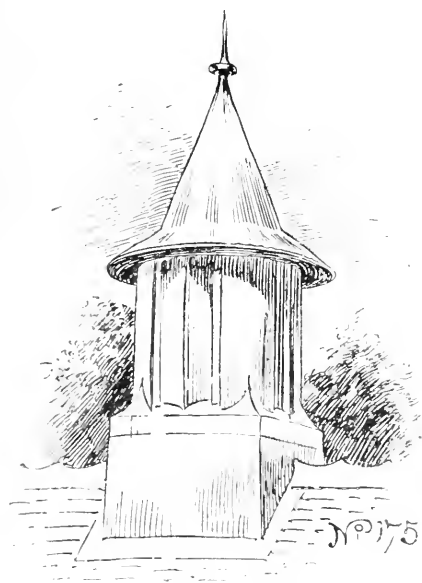
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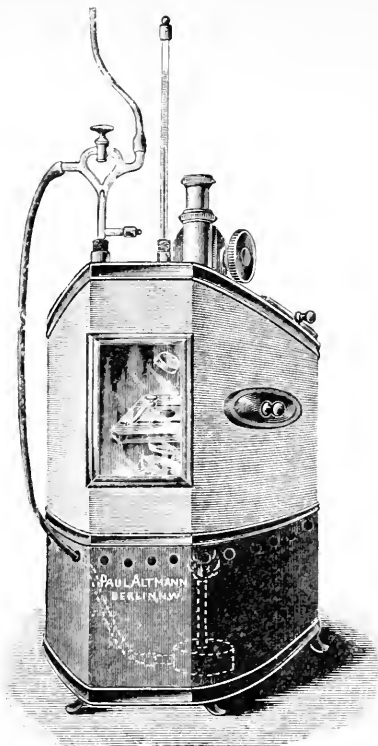
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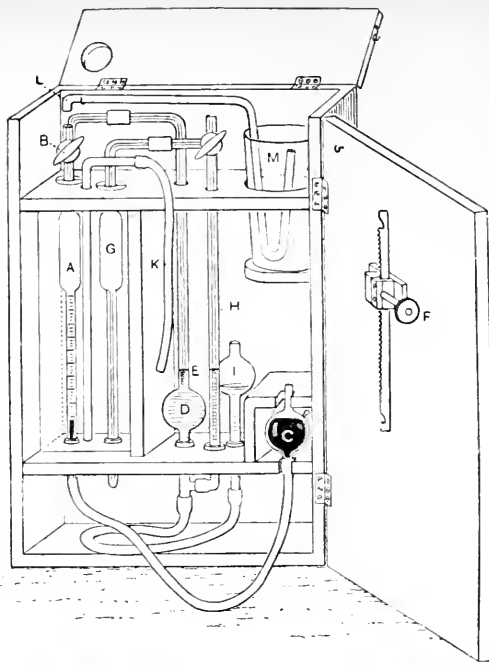
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